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The Carcinogenicity of *p*-Monomethylaminoazobenzene in Various Diets and the Activity of this Dye Relative to *p*-Dimethylaminoazobenzene*

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In studies on the carcinogenic activity of compounds related to *p*-dimethylaminoazobenzene it has been observed that *p*-monomethylaminoazobenzene is about as active as the dimethyl compound (10, 17). More recently Giese, Miller, and Baumann (4) showed that when these compounds were fed *ad libitum* at either of two levels for $2\frac{1}{2}$ to $3\frac{1}{2}$ months, the monomethyl compound always caused a higher incidence of hepatomas than the dimethyl compound. However, rats fed *p*-monomethylaminoazobenzene voluntarily consumed more diet and, therefore, more of the carcinogen. Other studies (13) demonstrated that *p*-dimethylaminoazobenzene was reversibly demethylated *in vivo* to *p*-monomethylaminoazobenzene, and that the latter compound appeared to be irreversibly demethylated to *p*-aminoazobenzene. Since both methylated compounds are present in the animal body when either dye is fed, it is difficult to determine which is the closer to the true carcinogen. The present studies were designed with this question in mind. The effect of various diets on the carcinogenicity of *p*-monomethylaminoazobenzene was determined, and a more rigid comparison was made of the activity of the monomethyl and dimethyl dyes.

The diets selected were of two types. In the first group were diets high in riboflavin or in which hydrogenated coconut oil was substituted for corn oil; these rations are known to inhibit the induction of tumors when *p*-dimethylaminoazobenzene is the carcinogen (7, 11, 12, 14). The other diets represented an attempt to affect the equilibrium *in vivo* between *p*-dimethylaminoazobenzene and *p*-monomethylaminoazobenzene by altering the concentration of methyl groups in the tissues of the rat. For this purpose guanidoacetic acid or nicotinamide was fed to deplete the stores of labile methyl; guanidoacetic acid is methylated in the liver to form creatine (1), while nicotinamide is excreted by the rat as N^1-methylnicotinamide (15). Choline was added to the diets as a source of labile methyl groups (16).

METHODS

Groups of young, mature, male rats of the Sprague-Dawley strain, 170 to 200 gm. in weight, were fed 0.056 per cent of *p*-monomethylaminoazobenzene or 0.060 per cent of *p*-dimethylaminoazobenzene (equimolar levels) for 13 to 14 weeks. A single group was fed 0.106 per cent of *p*-aminoazobenzene for 11 months. The animals were kept in groups of 5 to 8 in screen-bottom cages, and food and water were given *ad libitum* (see below for the conditions used in the paired-feeding experiment, Table IV). The dye was incorporated in the rations by dissolving it with heat in the fat of the diet. The rations were mixed in amounts sufficient for 3 to 4 weeks, and stored at 0° C. At the end of the dye-feeding period the livers were examined by laparotomy, and the rats were continued on the same diets without the dye for an additional 2 months. At this time the animals were killed for a final tumor count.

The constituents of the various diets are listed in Table I. The control diet was the synthetic ration used

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previously (14), except that no choline was added; Miner and his co-workers (14) demonstrated that this vitamin was not necessary for efficient tumor production when *p*-dimethylaminoazobenzene was fed. Each rat also received 1 drop of halibut liver oil monthly. Rations 3 and 10 contained 5 per cent of hydrogenated coconut oil in place of a like amount of corn oil, while diets 4 and 11 contained 10 and 20 mgm. respectively of riboflavin per kgm. rather than the 2 mgm. per kgm. incorporated in the other diets. Three-tenths or 0.5 per cent of choline chloride was added to diets 5, 8, and 12, while 0.35 per cent of nicotinamide or 0.30 per cent of guanidoacetic acid was included in diets cages and fed diets containing *p*-monomethylaminoazobenzene or *p*-dimethylaminoazobenzene (Table I, Diets 1 and 2) for 13 weeks. The food consumption of the rats of each pair was equalized throughout the experiment by restricting both to the voluntary consumption of the animal eating the least. The livers were examined by laparotomy at 13 weeks, and the animals continued on the control ration without the azo dyes. A second operation was performed on the members of each pair when a tumor was palpated in either of the rats. The surviving animals were killed for examination 9 weeks after the end of the dye-feeding period.

TABLE I: COMPOSITION OF DIETS *

Diet No.	Gm. per kgm.									
	1 and 9	2	3 and 10	4	5	6	7	8	11	12
Cerelose †	790	790	790	790	787	786	787	786	790	785
Casein (purified ‡)	120	120	120	120	120	120	120	120	120	120
Salts mixture	40	40	40	40	40	40	40	40	40	40
Corn oil	50	50		50	50	50	50	50	50	50
Hydrogenated coconut oil			50							
Choline chloride					3			3		5
Nicotinamide						3.5 §				
Guanidoacetic acid							3 §			
Riboflavin	0.002	0.002	0.002	0.010	0.002	0.002	0.002	0.002	0.020	0.002
p-Dimethylaminoazobenzene		0.60								
p-Monomethylaminoazobenzene	0.56		0.56	0.56	0.56	0.56	0.56		0.56	0.56
p-Aminoazobenzene								1.06		

* Each kgm. of diet contained also 3.0 mgm. thiamine chloride, 2.5 mgm. pyridoxine hydrochloride, and 7.0 mgm. calcium pantothenate.
† Pure glucose monohydrate obtained from Corn Products Refining Company.
‡ For preparation see reference 14.
§ The levels of methyl acceptors were lowered at the end of the dye-feeding period to 2.8 gm. nicotinamide and 2.9 gm. guanidoacetic acid per kgm. of diet.

6 and 7 respectively. If one assumes that the methyl group of *p*-monomethylaminoazobenzene is physiologically available, and that the only other methyl groups in the diet were furnished by 3.25 per cent of methionine in the casein (3), the nicotinamide was fed at a level equivalent to the methyl groups contained in the diet. On the same basis the guanidoacetic acid fed was equivalent to 87 per cent of the dietary methyl groups. These levels were chosen arbitrarily. If the methyl group from the azo dye is not completely available to the animal, then the proportion of acceptor to dietary methyl groups in these diets was larger than calculated. The rats on these levels of methyl acceptor lost weight, and accordingly after the carcinogen was removed from the diets at 14 weeks both compounds were fed at levels equivalent to 90 per cent of the methyl groups furnished by the methionine in the casein; *i.e.,* 0.28 per cent of guanidoacetic acid and 0.29 per cent of nicotinamide.

In another series 2 groups of Sprague-Dawley rats, paired by sex and weight, were kept in individual

In addition to the experimental groups described above other rats, which had received diets 1, 6, 7, or 12 for 2 to 16 weeks, were used for analytical purposes. These animals were kept in individual cages and fed 8 gm. of diet per day. At intervals rats from each group were killed and the content of azo dye in the blood and liver was determined. The method of analysis was essentially that described previously (9), except that 4 cc. each of 11 *N* potassium hydroxide, water, and 95 per cent ethanol were used for saponification in place of the proportions suggested in the original paper. Each liver was perfused *in situ* with 100 cc. of 2 per cent sodium citrate in order to remove as much blood as possible, and thus make the results more representative of the liver tissue.

Two of the compounds used were synthesized in this laboratory. The *p*-monomethylaminoazobenzene, prepared as described by Miller and Baumann (9), had a m. p. of 87-88° C.; the guanidoacetic acid, synthesized and reprecipitated according to Brand and Brand (2), had a decomposition point of 281-284° C.

RESULTS

In 10 of the 12 groups (Table II) the rats remained in good health, ate more than 10 gm. of diet per day, and gained 40 or more gm. during the dye feeding period. The rats receiving the methyl acceptors (Groups 6 and 7) consumed 9 gm. of ration per day, but began to lose weight after 3 months. Ninety-three to 100 per cent of the rats on each diet survived until the laparotomies were performed, and no more than 2 animals from any group died without tumors during the subsequent 2 month period on the dye-free rations.

The effect of hydrogenated coconut oil and riboflavin.—When 0.056 per cent of *p*-monomethylaminoazobenzene was fed for 14 weeks in the usual corn oil diet, the incidence of tumors at 22 weeks was 87 per cent (Table II, Group 1). Of the rats on hydrogenated coconut oil (Group 3) 31 per cent had hepatomas at 22 weeks, while the incidence of neoplasms in Group 4, which received 10 mgm. of riboflavin per kgm., was 58 per cent. When 0.056 per cent of *p*-monomethylaminoazobenzene was fed for only 13 weeks, 60 per cent of the rats on the corn oil diet (Group 9) developed neoplasms by 22 weeks, while 27 per cent of those receiving hydrogenated coconut oil (Group 10) had hepatomas at this time. However, only 7 per cent of the animals fed 20 mgm. of riboflavin per kgm. of ration (Group 11) had liver tumors by the end of the experimental period. Thus the substitution of hydrogenated coconut oil for corn oil definitely inhibited tumor induction due to *p*-monomethylaminoazobenzene. Increasing the level of riboflavin from 2 to 10 mgm. per kgm. reduced the number of hepatomas slightly, while a further increase to 20 mgm. per kgm. considerably inhibited tumor formation. Nevertheless, these dietary effects were less pronounced than those previously observed when *p*-dimethylaminoazobenzene was used as the carcinogen (7, 11, 12, 14). In general gross cirrhosis paralleled tumor incidence; it was most severe in the rats on the corn oil diet and less pronounced in those receiving either hydrogenated coconut oil or high levels of riboflavin.

TABLE II: CARCINOGENICITY OF *p*-MONOMETHYLAMINOAZOBENZENE IN VARIOUS DIETS

Group * no.	Dye fed	Diet	Dye feeding period, wks.	Average initial weight, gm.	Gain in weight 13-14 wks., gm.	Ave. food consumption, gm./rat/day	Survival † 13-14 wks.	Hepatomas ‡ 13-14 wks.	Hepatomas ‡ 22 wks.	Neg. survivors at 22 wks.	Gross cirrhosis at 13-14 wks. none-very mild	mild-mod.	severe
1	MAB §	Control	14	185	58	11.5	15/15	6/15	13/15	1	1	9	5
2	DAB §	Control	"	183	42	10.4	15/15	1/15	9/15	4	2	12	1
3	MAB	HCNO ‖	"	188	59	13.3	13/13	1/13	4/13	9	8	5	0
4	"	10 mgm. riboflavin/kgm.	"	187	66	12.1	12/12	2/12	7/12	3	3	8	1
5	"	0.3% choline	"	182	63	10.9	14/15	8/14	12/14	1	5	9	1
6	"	0.35% nicotinamide	"	186	— 11	9.6	15/15	5/15	12/15	1	0	0	15
7	"	0.3% guanidoacetic acid	"	189	4	9.0	14/15	2/14	9/14	3	0	5	10
8	AB §	0.3% choline	"	182	67	13.0	12/12	0/12	0/12 **	7	12	0	0
9	MAB	Control	13	186	51	11.4	15/15	1/15	9/15	5	2	8	5
10	"	HCNO	"	185	65	12.3	15/15	1/15	4/15	9	5	10	0
11	"	20 mgm. riboflavin/kgm.	"	179	83	13.4	14/15	0/14	1/14	13	8	6	0
12	"	0.5% choline	"	186	53	11.7	15/15	3/15	11/15	3	6	9	0

* Groups 1 to 8 constitute one series of experiments and are directly comparable; Groups 9 to 12 comprise a second series.
† Survival = number living over number at start.
‡ Hepatomas = number with hepatomas over number alive at time of operation.
§ DAB = *p*-dimethylaminoazobenzene.
MAB = *p*-monomethylaminoazobenzene.
AB = *p*-aminoazobenzene.
‖ HCNO = hydrogenated coconut oil.
** This group was fed the dye continuously for 11 months; no tumors were noted even at this time.

The effect of methyl donors or acceptors.—In the presence of the azo dye the most obvious effects of choline, as a methyl donor, and of guanidoacetic acid or nicotinamide as methyl acceptors, were upon the degree of liver damage. Choline tended to minimize the gross cirrhotic changes normally appearing when *p*-monomethylaminoazobenzene is fed to rats (Table II, Group 5 versus Group 1; Group 12 versus Group 9). This is in agreement with the observations of White and Edwards (18), who fed *p*-dimethylaminoazobenzene with or without 0.2 per cent of choline in a diet containing 6 per cent of casein and 0.5 per cent of cystine.

The methyl acceptors, on the other hand, tended to aggravate the cirrhotic condition. The livers of rats fed *p*-monomethylaminoazobenzene and either guanidoacetic acid or nicotinamide (Table II, Groups 6 and 7) were very much more cirrhotic after 14 weeks

than those of the rats receiving the azo dye alone (Group 1). Furthermore, when the corresponding rations without the azo dye were fed during the subsequent 8 weeks the cirrhosis receded, and the livers of Group 1 tended to resume a smooth, normal appearance. However, in the presence of nicotinamide or guanidoacetic acid the cirrhosis did not regress appreciably, even after 8 weeks on the dye-free diets. The amounts of nicotinamide and guanidoacetic acid fed were about the maximum that can be tolerated by adult rats given the methyl acceptor and carcinogen simultaneously. The animals in both groups gained about 20 gm. during the first 4 weeks and remained in fair health for the next 8 to 10 weeks. Thereafter some of the rats, especially those fed nicotinamide, began to lose weight. Removing the carcinogen at 14 weeks and reducing the amount of methyl acceptors slightly (see Methods) permitted survival for the following 2 months.

But in spite of their effects on the degree of liver damage, neither the methyl donor nor the methyl acceptors altered greatly the number of tumors caused by the ingestion of *p*-monomethylaminoazobenzene. Thus while 87 per cent of the rats receiving no supplement (Table II, Group 1) had hepatomas at 22 weeks, the incidence was 86 per cent when 0.3 per cent of choline chloride (Group 5) was added to the diet, 80 per cent when 0.35 per cent of nicotinamide (Group 6) was included, and 64 per cent with 0.3 per cent of guanidoacetic acid (Group 7) in the diet. When the level of choline chloride was raised to 0.5 per cent and the carcinogen was fed for only 13 weeks, 73 per cent of the rats (Group 12) had neoplasms at 22 weeks as compared to an incidence of 60 per cent in the unsupplemented group (Group 9).

The methyl acceptors and methyl donor also failed to alter appreciably the amounts of azo dye in the blood and livers of rats fed *p*-monomethylaminoazobenzene (Table III). The blood of rats that had received these diets for 2 weeks contained an average of 15 to 18 μgm. of *p*-aminoazobenzene per cc.; no more than traces of the methylated dyes were detected. The livers of these rats contained 2 to 3 μgm. of *p*-dimethylaminoazobenzene, 2 to 5 μgm. of *p*-monomethylaminoazobenzene, and 4 to 8 μgm. of *p*-aminoazobenzene; there was no significant difference between the values for the rats fed the control diet or high levels of choline, guanidoacetic acid, or nicotinamide for only 2 weeks. When the same diets were fed for 10 to 16 weeks, the *p*-aminoazobenzene content of the blood again averaged 15 to 18 μgm. per cc., and the level of the dimethyl compound in the liver was the same as at 2 weeks: 2 to 3 μgm. per liver. The amount of *p*-aminoazobenzene in these livers was slightly higher, 8 to 9 μgm. per liver, than at 2 weeks, possibly because these livers were cirrhotic and there was less assurance that the perfusion had been complete; any blood not removed would raise the apparent content of *p*-aminoazobenzene in the organ. The amount of the monomethyl compound in the livers increased to an average of 6 μgm. for the rats fed either guanidoacetic acid or nicotinamide, whereas the livers of the rats on the control diet for the same period contained only 2.5 μgm. Again the cirrhosis, with a consequent decrease in normal liver tissue, may have been responsible for the difference.

p-Aminoazobenzene and choline.—Feeding 0.3 per cent of choline chloride with 0.106 per cent of *p*-aminoazobenzene did not appear to promote the formation of liver tumors by this dye. Of the 12 rats fed the compound continuously 5 survived for 11 months, but neither tumors nor gross cirrhosis were found. Analyses of tissues from 2 of the rats fed the diet for 11 months showed the presence of appreciable amounts of *p*-aminoazobenzene, but neither of the methylated dyes could be detected. On the average there were 18 μgm. per cc. of blood, 22 μgm. per liver, and 9 μgm. per gm. of perirenal fat.

While other investigators (6, 10) have also failed to induce neoplasms with *p*-aminoazobenzene, Kirby (8) produced liver tumors (2 macroscopic, 1 microscopic) in 3 of 16 rats by feeding 0.2 to 0.3 per cent of this compound (3.8 to 5.5 times the molar level used for the dimethyl compound) for 17 months. His conclusion that ". . . methylation of the primary amine, *p*-aminoazobenzene, enhances but does not initiate carcinogenic activity . . ." may be valid, but other considerations should be mentioned. If small amounts of *p*-monomethylaminoazobenzene or *p*-dimethylaminoazobenzene either contaminated the dye fed, or were formed *in vivo,* the quantities in the tissues might have been sufficient to cause tumors in such a long experiment. Maruya (6) reported 2 hepatomas after feeding 0.006 per cent of *p*-dimethylaminoazobenzene for 371 days, but none from 0.002 per cent in 400 days. If 1 to 2 per cent of the *p*-aminoazobenzene fed at the 0.2 per cent level were methylated *in vivo,* this would be equivalent to feeding 0.002 to 0.005 per cent of the methylated dyes in the diet. It would be very difficult to detect analytically such small amounts of one dye in the presence of 50 to 100 times as much of another derivative. For example, in the present experiment the livers of the rats fed 0.106 per cent of *p*-aminoazobenzene (Group 8) contained 22 μgm. of the dye; the chromatographic detection of bands containing 0.2 to 0.4 μgm. (1 and 2 per cent of the total) of either of the two methylated dyes in the presence of this amount of *p*-aminoazobenzene is very uncertain with the present methods.

Relative carcinogenicity of p-dimethylaminoazoben-

zene and p-monomethylaminoazobenzene.—In agreement with previous results from this laboratory (4) the rats fed *p*-monomethylaminoazobenzene *ad libitum* developed more tumors than those on the dimethyl compound. The incidences were 40 and 87 per cent for the monomethyl dye at 14 and 22 weeks, and 7 and 60 per cent for the animals receiving *p*-dimethylaminoazobenzene (Table II, Groups 1 and 2). Under paired-feeding conditions, however, these compounds had equal activity (Table IV). Of the 15 pairs started both members of 13 pairs survived until at least 1 had a hepatoma. Two rats on each compound had liver tumors at 13 weeks, and at 22 weeks the total number was 8 for the rats fed *p*-dimethylaminoazobenzene and 9 for the group receiving the monomethyl compound. There was no significant difference in the rate of

TABLE III: LEVELS OF *p*-MONOMETHYLAMINOAZOBENZENE AND ITS METABOLITES IN TISSUES OF RATS FED THIS DYE WITH CHOLINE OR METHYL ACCEPTORS

Diet	Period dye was fed	No. of rats/ group	Blood AB *	Liver (perfused) DAB *	Liver (perfused) MAB *	Liver (perfused) AB
	wks.		μgm./cc.†	μgm. per liver †		
Control	2	3	18.3 (14.2-22.7)	3.0 (2.1-4.0)	2.7 (1.9-3.5)	6.2 (4.3-8.1)
0.3% Guanidoacetic acid	"	"	15.6 (15.4-16.0)	2.7 (2.2-3.1)	3.8 (2.4-4.6)	4.8 (3.9-5.5)
0.35% Nicotinamide	"	"	16.5 (15.2-17.8)	2.0 (1.8-2.4)	3.5 (2.6-3.9)	4.8 (4.3-5.2)
0.5% Choline	"	"	14.2 (11.2-17.5)	2.0 (1.9-2.1)	4.5 (4.3-4.8)	7.2 (6.3-8.9)
Control	10-16	5	15.2 (8.1-20.8)	2.7 (1.4-3.7)	2.5 (1.4-3.0)	8.3 (6.1-9.3)
0.3% Guanidoacetic acid	"	"	17.1 (13.8-20.6)	1.9 (1.2-3.3)	5.7 (3.0-10.4)	9.2 (6.1-12.3)
0.35% Nicotinamide	"	"	18.2 (12.8-22.0)	1.9 (1.2-3.0)	6.8 (4.6-8.6)	8.8 (7.5-9.7)

* DAB = *p*-dimethylaminoazobenzene.
MAB = *p*-monomethylaminoazobenzene.
AB = *p*-aminoazobenzene.
† The single value is the average for the group; figures in parentheses indicate the ranges found.

TABLE IV: THE RELATIVE CARCINOGENICITIES OF *p*-DIMETHYLAMINOAZOBENZENE AND *p*-MONOMETHYLAMINOAZOBENZENE AS DETERMINED BY PAIRED-FEEDING *

Pair no.	Sex	Average initial weight, gm.	Average weight increment, gm.	Dye consumed in 13 weeks, millimols/rat	*p*-Monomethylaminoazobenzene Hepatoma, 13 wks.	*p*-Monomethylaminoazobenzene Hepatoma, 22 wks.	*p*-Monomethylaminoazobenzene Gross cirrhosis, 13 wks.	*p*-Dimethylaminoazobenzene Hepatoma, 13 wks.	*p*-Dimethylaminoazobenzene Hepatoma, 22 wks.	*p*-Dimethylaminoazobenzene Gross cirrhosis, 13 wks.
1	M	205	22	2.12	0	0	normal	0	0	normal
2	F	162	14	1.89	0	+	mild	0	+	moderate
3	F	172	— 19	1.76	0	+	moderate	0	+	severe
4	M	181	0	1.72	0	+	severe	0	+	normal
5	M	167	8	2.05	0	+	moderate	0	+	severe
6	M	178	30	2.07	0	+	normal	0	+	mild
7	M	216	— 9	1.95	+	+	moderate	0	0	normal
8	F	184	— 14	1.86	+	+	mild	0	0	mild
9	F	164	— 19	1.92	0	+	severe	0	0	normal
10	M	168	17	1.98	0	+ †	moderate	0	? †	mild
11	M	201	— 24	1.99	0	? ‡	moderate	+	+	mild
12	F	160	7	1.89	0	0	mild	+	+	moderate
13	M	202	— 30	1.80	0	0	normal	0	+	moderate
Average	..	182	— 1	1.93	2/13	9/13	....	2/13	8/13	....

* Two pairs in which 1 rat died before either animal developed a tumor are not included in this table.
† In this pair the rat receiving *p*-monomethylaminoazobenzene had a liver tumor when the animal on *p*-dimethylaminoazobenzene died after 18 weeks on the experiment.
‡ Died after 14 weeks.

formation or growth of the neoplasms on the two diets; likewise the cirrhosis at the end of the dye-feeding period was generally mild to moderate in both cases. Further, there was no correlation between the amount of dye consumed by a given rat and the rate of tumor formation. While these data demonstrated that the two compounds had equal carcinogenic potency as determined by the average of 13 pairs of rats, they also indicated the heterogeneity of such rats with respect to susceptibility to the induction of hepatomas by the azo dyes. Both members of 1 pair were tumor-free at 22 weeks and both members of 5 pairs had tumors at this time. In 7 of the 13 pairs, however, only 1 rat of each pair had developed a neoplasm by the end of the experimental period: 4 on the monomethyl compound and 3 on *p*-dimethylaminoazobenzene.

While the rats remained in generally good health, the average weight change was −1 gm. (Table IV) during the dye feeding period, in contrast to gains of 42 and 58 gm. for the rats fed *p*-dimethylaminoazobenzene and *p*-monomethylaminoazobenzene *ad libitum* (Table II). This difference was probably due to the fact that both rats of each pair were continually restricted to the lower voluntary food consumption of the two, so that both animals were penalized if either went off feed for a few days. The average food intake was 8 gm. per rat per day.

DISCUSSION

The results of these experiments cannot be interpreted as favoring either *p*-dimethylaminoazobenzene or *p*-monomethylaminoazobenzene as being closer to the true carcinogen. The equal activity of these compounds, when compared under controlled feeding conditions, suggests either that both compounds are carcinogenic or that they are both converted at the same rate *in vivo* to the active compound. Or, if only one of these two dyes is the actual carcinogen, then the rate of conversion of the noncarcinogenic one to the active compound must be very efficient indeed. On the other hand the greater effect of such dietary constituents as hydrogenated coconut oil or riboflavin on carcinogenesis due to the dimethyl dye, as compared to *p*-monomethylaminoazobenzene, might be interpreted as indicating that the latter compound was nearer to the true carcinogen. However, it is more likely that this is another expression of the difference between *ad libitum* and controlled feeding with rats receiving the monomethyl compound. Under identical conditions it is doubtful if any difference in the inhibition by riboflavin and hydrogenated coconut oil would be observed when the two dyes were compared. Incidentally, it is still not clear why rats fed *p*-monomethylaminoazobenzene voluntarily consume more food than those receiving equimolar amounts of the dimethyl dye in the diet.

Since *p*-dimethylaminoazobenzene and *p*-monomethylaminoazobenzene are formed from each other *in vivo,* it is quite possible that a transmethylation reaction may be involved. However, the conversion of *p*-monomethylaminoazobenzene to *p*-aminoazobenzene is not reversible to any detectable extent, and part of or all the monomethyl compound may be degraded by a mechanism that does not make the methyl group available to other molecules such as guanidoacetic acid or nicotinamide. Liver preparations, for instance, have been observed to demethylate sarcosine by oxidation of the methyl group to formaldehyde (5). If the methylated aminoazo dyes undergo a similar degradation, feeding large quantities of methyl acceptors with *p*-monomethylaminoazobenzene would probably not alter the rate of formation of the completely demethylated compound. The inability to demonstrate a difference in the quantity of the dimethyl compound in the liver may be due also to the difficulty of altering the methyl balance of animal tissues sufficiently to affect a given reaction to a noticeable extent.

SUMMARY

1. Hepatomas developed by 22 weeks in 60 to 87 per cent of rats fed a synthetic diet containing corn oil and *p*-monomethylaminoazobenzene for 13 to 14 weeks, whereas only 30 per cent of the rats fed hydrogenated coconut oil developed tumors by this time. Raising the riboflavin content of the ration from 2 to 10 mgm. per kgm. reduced the tumor incidence slightly; when 20 mgm. per kgm. were fed, only 1 of 14 rats had a hepatoma at 22 weeks.

2. Rats receiving diets that contained 0.3 per cent of guanidoacetic acid, 0.35 per cent of nicotinamide, or 0.3 or 0.5 per cent of choline with *p*-monomethylaminoazobenzene developed approximately the same number of neoplasms as the animals on the control diet, although the methyl acceptors caused a more severe gross cirrhosis than the control diet, whereas the addition of choline minimized the cirrhosis. Analyses of the livers and blood from rats on each of these diets indicated that the methyl acceptors and donor did not alter greatly the levels of *p*-dimethylaminoazobenzene, *p*-monomethylaminoazobenzene, and *p*-aminoazobenzene in the tissues.

3. No liver tumors or gross cirrhosis were found in rats fed 0.106 per cent of *p*-aminoazobenzene with 0.3 per cent of choline for 11 months.

4. When *p*-dimethylaminoazobenzene and *p*-monomethylaminoazobenzene were fed *ad libitum,* the rats receiving the monomethyl compound developed more tumors than those on the dimethyl dye. However,

when the two compounds were compared by the paired-feeding technic the carcinogenic activities were found equal.

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Calcium, Copper, and Zinc in the Epidermal Carcinogenesis of Mouse and Man*

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The role of the minerals in epidermal methylcholanthrene carcinogenesis in mice has been described (1), and the integration of the chemical, physical, and histological changes has been reviewed recently by Cowdry (3).

The present study was undertaken to compare the calcium, copper, and zinc content of mouse and human epidermis with that in squamous cell carcinomas from mouse and man respectively. Previous studies have shown that these metals are low in mouse epidermis rendered hyperplastic by methylcholanthrene, and very low in a transplantable squamous cell mouse carcinoma (1). The potassium, sodium, calcium, and magnesium content of normal human epidermis has been reported (4).

EXPERIMENTAL PROCEDURE

The procedure for separating epidermis from dermis and the analytical methods for calcium, copper, and zinc have been given in previous papers (1). Because human squamous cell carcinomas of sufficient size for analysis are rare we had to be content with 5 samples, which were gathered over a rather long period of time. However, the analyses of these showed significant differences from those of normal human epidermis, and the differences were found in all specimens. The carcinomas were cleared of blood and connective tissue and only the solid pieces were used for analysis, since necrotic tissue affects considerably the calcium content (2). Calcium determinations on all samples were done in duplicate, a practice employed also for copper and zinc when sufficient tissue was available. Seven specimens of normal human epidermis were analyzed for copper and zinc, and many samples for the alkalies and alkaline earths (4).

RESULTS

The results of analyses of normal mouse and human epidermis, mouse epidermis treated with benzene or methylcholanthrene, and mouse and human squamous cell carcinomas are shown in Table I. Only calcium, copper, and zinc were determined in the squamous cell carcinomas because of the scarcity of sufficient material. Data on these three metals are of especial interest, for they are significantly altered in epidermal carcinogenesis in mice (1). The results are expressed as micrograms of metal per 100 mgm. of epidermis. The calcium content of normal mouse epidermis is

TABLE I: MINERAL COMPOSITION OF EPIDERMIS

	Micrograms of metal per 100 mgm. epidermis					
Material	K	Na	Ca	Mg	Zn	Cu
MOUSE						
Normal	347	168	44.0	19.0	5.2	0.58
Benzene-treated	351	163	42.0	19.0	5.5	0.58
Hyperplastic	346	141	19.0	22.6	3.8	0.33
Carcinoma	326	141	9.0	18.0	1.7	0.10
HUMAN						
Normal	322	123	16.0	18.0	2.4	0.54
Carcinoma	...	...	8.5	...	1.7	0.16

nearly three times higher than that of normal human epidermis, whereas the copper content of both is nearly the same. The zinc content of mouse epidermis is more than twice that of the human. The calcium content of hyperplastic mouse epidermis is 57 per cent less than normal, but only slightly greater than that of normal human epidermis. Also a comparison of hyperplastic mouse epidermis with normal human epidermis shows that the magnesium content of both is fairly similar, whereas the potassium and sodium content of the former is respectively about 6 and 13 per cent higher than that of the latter. In other words, methylcholanthrene-treated mouse epidermis and normal human epidermis are closely similar chemically as far as these metals are concerned. They are also somewhat alike histologically.

The calcium, zinc, and copper content of transplantable squamous cell carcinomas of the mouse induced by methylcholanthrene is respectively 80, 67, and 83 per cent less than that of untreated mouse epi-

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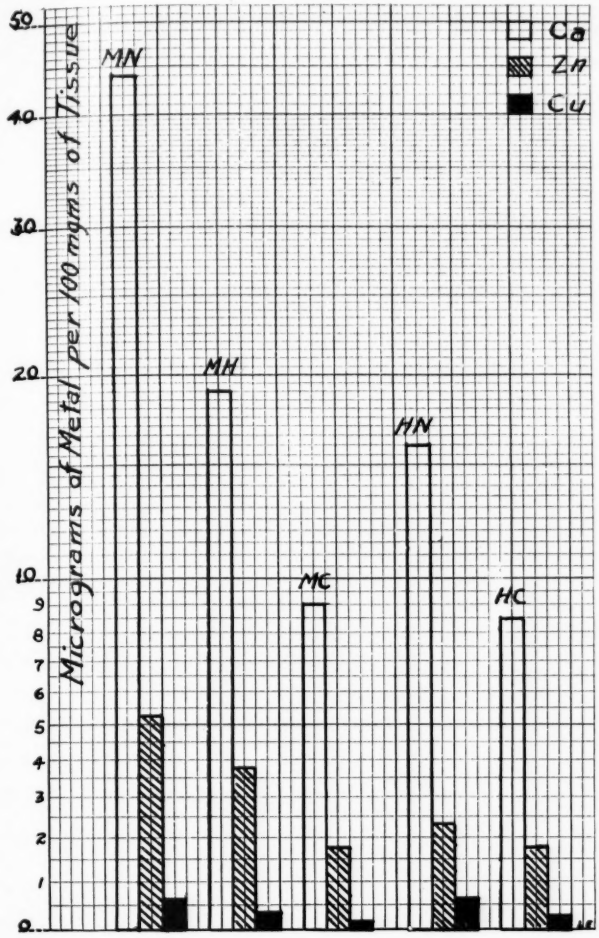

FIG. 1.—Calcium, zinc, and copper in epidermal carcinogenesis in mouse and man. The data are plotted on semi-logarithmic paper, and the letters denote the following: MN—normal mouse epidermis; MH—hyperplastic mouse epidermis; MC—mouse transplantable squamous cell carcinoma; HN—normal human epidermis; HC—human squamous cell carcinoma.

dermis. On the other hand, the calcium, zinc, and copper content of human squamous cell carcinomas is respectively 47, 30, and 70 per cent less than that of normal human epidermis. Except for the pronounced decrease in copper, the diminution in calcium and zinc is not nearly so great as in the mouse carcinoma, although the amount of calcium is nearly the same in both. The possible implications of the changes in these metals during epidermal carcinogenesis in mice have been discussed (1).

The conspicuous alterations in calcium, copper, and zinc in carcinogenesis in mouse and man is more clearly illustrated in Fig. 1. These results show that the changes in these metals in the epidermal carcinogenesis of mouse and man are similar.

SUMMARY

The change in concentration of calcium, copper, and zinc in epidermal carcinogenesis in mouse and man is briefly outlined. These studies indicate that the process occurring in both species is similar, in that there are considerable decreases in the amounts of these metals when normal mouse epidermis is transformed into squamous cell carcinoma by methylcholanthrene, and when the content of these metals in normal human epidermis and in human squamous cell carcinomas is compared.

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Carcinogens and the Regeneration Patterns After Injury

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INTRODUCTION

There is considerable evidence that carcinogens injure cells. Pullinger (10) described degenerative changes and believed that the injury was specific. Cramer and Stowell (2) found that carcinogens did not induce a proliferation of epithelial cells, but injured them and inhibited their mitotic activity for several days. The subsequent epithelial proliferation suggested to them a "protective response."

Orr (8) described not only an initial atrophy of epithelium after the first application of a carcinogen, but changes in the collagen fibers of the dermis that he believed altered the blood supply of the epithelial cells and caused the malignant change.

Wolbach (21) pointed out that cells in the subcutaneous tissues were destroyed about buried cholesterol pellets containing a carcinogen, and that encapsulation did not occur properly. Orr (9) confirmed these observations, and described the formation of fibrous tissue around paraffin pellets containing a carcinogen as "deficient in amount and failing to condense properly into a firm capsule." Stewart (18) wrote that the alteration about buried cholesterol pellets containing a carcinogen was a focal and diffuse chronic inflammatory change.

If carcinogens produce neoplasms by causing cell injury, then to know what happens to the regeneration patterns that usually follow injury should help in understanding how tumors form. These regeneration patterns, epithelization and fibroplasia, are quite different in nature. Epithelization results from the amoeboid motion, proliferation, and maturation of preexisting epithelial cells; the process restores surfaces. Fibroplasia, on the other hand, which restores the continuity of the deeper structures of the body, consists of the proliferation and maturation of fibroblasts and the formation of reticulin and collagen. In fact, the phase of proliferation is brief, and the greater portion of the pattern is concerned with the elaboration of intercellular substances.

This investigation will compare the changes that carcinogens cause in epithelium recovering from a traumatic injury and in intact epithelium. By observing fibroplasia about a buried carcinogen when a tumor develops and when a tumor fails to develop in (a) a susceptible animal and in (b) a naturally resistant animal, changes leading to the production of a sarcoma or to encapsulation should be discernible. Lastly, changes in fibroplasia about an embedded carcinogen when a sarcoma forms will be compared with those taking place in dermis when a epithelioma forms.

METHODS

Silk thread was used to implant the carcinogen because methylcholanthrene could be easily loaded into its interstices, and the reactions of tissues to silk as a foreign body have been extensively studied before. (5) For a chemical control a benzene ring compound, similar but not carcinogenic, was loaded into silk thread. Upon the advice of Dr. Fieser, of Harvard University, 1,2-benzanthracene was used for this purpose. No attempt was made to place a quantitative amount of either chemical into a definite length of thread, but instead C-silk was impregnated with all the chemical that the strand could possibly hold. To do this, an excessive amount of methylcholanthrene or 1,2-benzanthracene was placed in a test tube and a length of thread was curled up into a ball and inserted into the tube on top of the powder. The tube was then heated until the powder melted and the thread was stirred in the liquid while re-crystallization took place. Melting and stirring was done 2 or 3 times, until the thread had become swollen with the chemical and was of an even yellow color. Threads prepared in this way were stored in sterile petri dishes.

These threads were buried in the muscles of rats of the Wistar strain and mice of the RIII strain.[1] Guinea pigs were used as the species of animal relatively resistant to the action of methylcholanthrene.

The threads were placed in the following manner. Under general anesthesia a small incision was made in the skin under aseptic technic and about 2 cm. of thread were inserted by means of a curved needle in the underlying muscle. Sometimes the muscles of the abdominal wall were employed, and at other times

[1] Department of Cancer Research, Columbia University.

those of the back. The skin was then sutured. Later, particularly when threads were being placed in the muscles of the back, 2 small nicks were made in the skin at the required distance apart and the needle was made to enter and leave through these openings. With this technic no suturing of the skin was required.

In some animals the thread containing the carcinogen and the control thread were inserted in different muscles in the same animal, while in other instances the control and experimental threads were placed in different animals.

The rats and mice were fed on equal parts of Purina dog chow and Rockland rat pellets, the guinea pigs on hay, oats, and greens, and all received water *ad libitum*. They were killed at various intervals; approximately 10, 25, 50, 75, 90, 120, 160, and 240 days. Two animals were employed for each day. At the last 4 intervals, animals were killed and the tissues examined even though tumors had not appeared.

When an animal was killed the muscle containing the thread was excised in a block and fixed in Bouin's solution. Forty-eight hours later each block was cut at right angles to the long axis of the thread to obtain a cross section for microscopic study.

After experimenting with all the modifications of the silver technic for demonstrating collagen and reticulin fibers; with Mallory's phosphotungstic blue; Masson's trichome; and Van Gieson's stain, the last named was finally employed without counter-stain because it gave the best tinctorial picture of the aging of reticulin and collagen. Although there has been much controversy about the value of staining methods for reticulin and collagen, certainly there can be little disagreement with the observation that about 1 week after new reticulin fibrils are laid down they no longer take the yellow of picric acid in Van Gieson's stain, but the red of acid fuchsin instead. Hematoxylin-eosin stains were employed to study cell morphology.

The effect of the carcinogen on the rate of proliferation of epithelium was measured by placing the chemical on a growing island of skin left in the center of a wound on the tail of a rat. The need of measuring extension of epithelium in an object free of contraction has been reported elsewhere (6). The defect was dressed daily with 0.3 per cent methylcholanthrene in benzene, followed by vaseline; a solution of this strength is known to produce epithelial cancer. Control islands were dressed with benzene only, followed by vaseline. The amount of extension was measured every day for 2 weeks with a calibrated eye piece on a microscope. Three readings were averaged. Sections were cut from these growing islands for microscopic study.

A solution of 0.3 per cent methylcholanthrene was painted on the skin of the mouse at weekly intervals to produce the changes in the intact skin. The changes in both the epithelium and the connective tissue of the derma were studied microscopically at intervals similar to those employed for the buried carcinogen. One animal was used for each observation in these experiments.

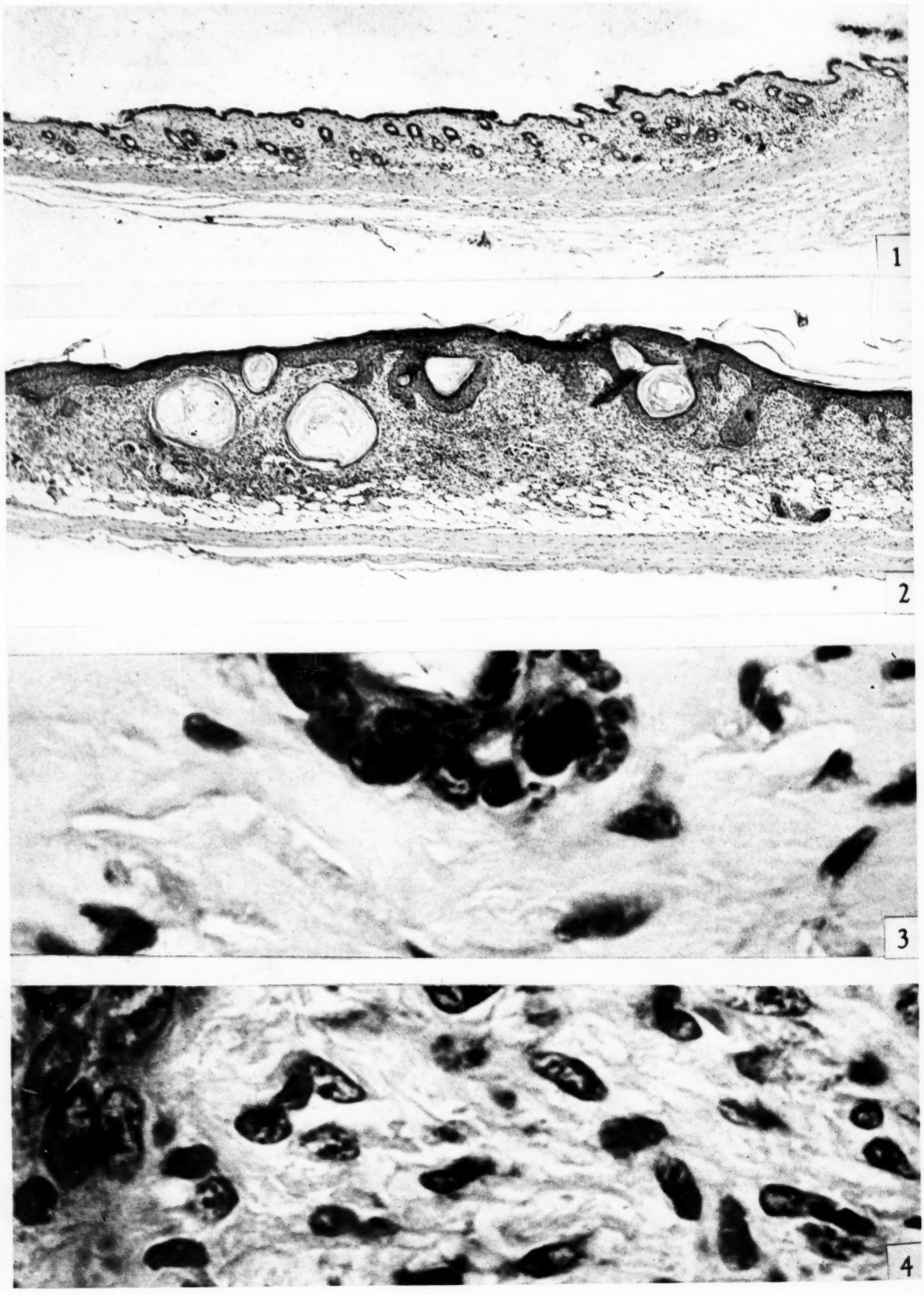
RESULTS

After the first application of methylcholanthrene to the intact skin of the mouse, the epithelium was injured and degenerated; that around the hair follicles almost disappeared. Keratin was lost, and in some animals only the basement layer and an additional layer of epithelium remained. Epilation occurred after 7 to 10 days.

Two weeks later the entire skin appeared thicker than normal (Figs. 1 and 2). In the epidermis more layers were visible, and the cells were larger. The epithelium in the hair follicles showed the same changes. Subsequent applications of the carcinogen did not cause their degeneration to take place again, but instead the epithelium remained hypertrophied and hyperplastic.

Two weeks after the first application of methylcholanthrene the dermis was thickened; its collagen fibers were swollen and the spaces between them were larger. They were interspersed with many mononuclear cells and with numerous spindle-shaped fibroblasts (Fig. 2). With repeated applications the large collagen fibers gradually disappeared and were finally entirely replaced by a very cellular connective tissue with thin, widely spaced reticulin fibrils. After 13 to 20 weeks these reticulin fibrils were not oriented to the surface of the skin like the original collagen fibers, and there were many more mononuclear cells present than are usually found in connective tissues. With the passage of time these fine fibrils did not differentiate into large fibers, nor did they become more numerous as they do after a traumatic injury (Figs. 3 and 4). As this change neared completion, papillomas began to appear on the surface of the epithelium, as well as irregular and frequent downgrowths of epithelium. These downgrowths contained cells varying in size and shape and undergoing mitosis.

The rate of epithelization under the influence of the carcinogen did not differ from the rate when no carcinogen was applied. The average rate of epithelization in the benzene control was 0.5 mm. a day, while under the influence of methylcholanthrene it varied from 0.38 to 0.41 mm. a day. Microscopically no difference could be found between the epithelial cells extending over the wound treated with the carcinogen and those extending over the wound treated with benzene. In both instances the cells in the old epithelium were hypertrophic, more layers were visible,



FIGS. 1-4

and those in the basal layer were more numerous and cuboidal (Fig. 5). Mitotic figures were about equal in number in both wounds.

Before describing how the carcinogen changes the process of fibroplasia, the normal pattern of encapsulating a silk thread and a thread containing the noncarcinogenic chemical will be detailed.

Encapsulation of Silk Thread

After a silk thread had been inserted in muscle a few leukocytes appeared around it for the first 2 or 3 days. By the fifth day only mononuclear cells could be found, infiltrating between the fibers of silk and surrounding the strand. Fibroblasts appeared within and about the strand on the fourth or fifth day. By the 14th day, encapsulation was usually complete. Each fiber of silk was encased in a separate sheath of red-staining reticulin as well as the periphery of the strand. Fibroblasts were already fewer in number and they were definitely spindle-shaped. Multinucleated giant cells were present in some locations between the fibers, and occasionally in the capsule. The capsule was a thin ring of reticulin about the circumference of the strand.

Variations in this pattern have been observed; sometimes exudation was more extensive, and the leukocytic phase lasted a day or two longer. Usually when the leukocytic phase persisted longer, a heavier capsule was deposited about the strand and later was gradually reabsorbed.

Encapsulation of Thread Containing 1,2-Benzanthracene

The inclusion of the noncarcinogenic chemical in the silk thread caused more exudation; leukocytes infiltrated between its fibers and surrounded its periphery. They remained several days longer than about the untreated thread and, correspondingly, fibroblasts appeared later within the interstices of the thread and reticulin formation was delayed and more abundant. By the 23rd day, however, or 9 or 10 days later than in the controls, encapsulation was complete. The fibroblasts were spindle-shaped or mature, and most of the reticulin fibrils took the red of the Van Gieson's stain (Figs. 6 and 7). Immediately about the circumference of the thread the reticulin fibrils were arranged circularly and regularly. The layer was slightly thicker than in the controls. Either healthy muscle lay immediately adjacent to this newly formed capsule, or sometimes there existed a small band of mononuclear cells separating the capsule from the healthy muscle. Fibroblasts appeared later in this band, and reticulin fibrils were deposited to form a thick capsule. This variation usually occurred when the individual fibers of silk were not encapsulated. After each individual silk fiber was completely surrounded by reticulin, the capsule again narrowed. This variation was complete in about 30 days.

Reaction of Tissues to Thread Containing a Carcinogen

By contrast, a striking alteration took place in the encapsulation of a thread impregnated with methylcholanthrene. A wide area of destruction of muscle took place about the thread (Fig. 8). Cells, both leukocytic and monocytic, surrounded the strand and infiltrated between its fibers. Many of the cells between the fibers had been killed, since only "shadow forms" could be seen. In fact, destruction of cells within the thread continued for a long period of time; for at 150 days (Fig. 9), and even later, after sarcomas had developed, at 240 days, either no cells or only remnants of them could be found between the fibers of the carcinogenic thread. For a long period of time, also, an amorphous pink-staining material could be identified between the fibers of the thread. Methylcholanthrene stains in this manner.

About the thread the cells of the exudative reaction remained in a wide irregular zone. They could always be seen at a distance from the thread, again suggesting that those near the strand were destroyed. This "halo" of cells was so clearly visible that the slides of the car-

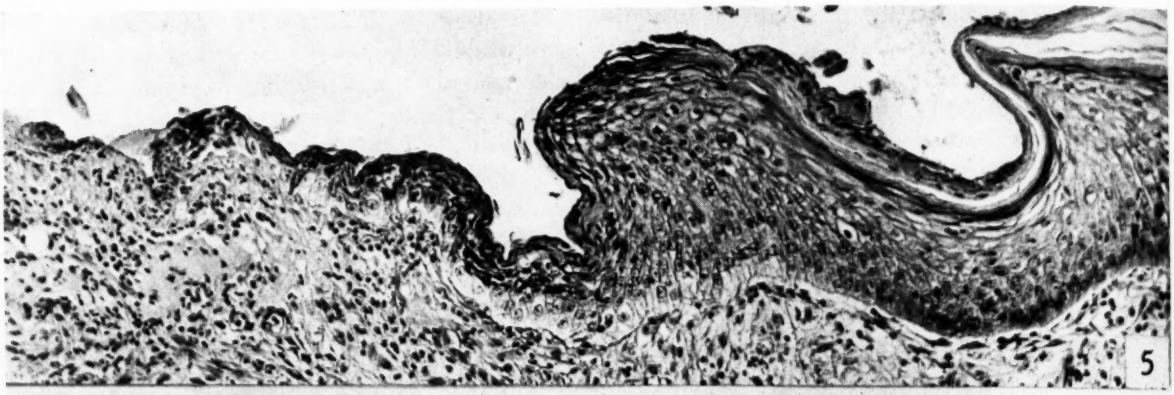
DESCRIPTION OF FIGURES 1 TO 4

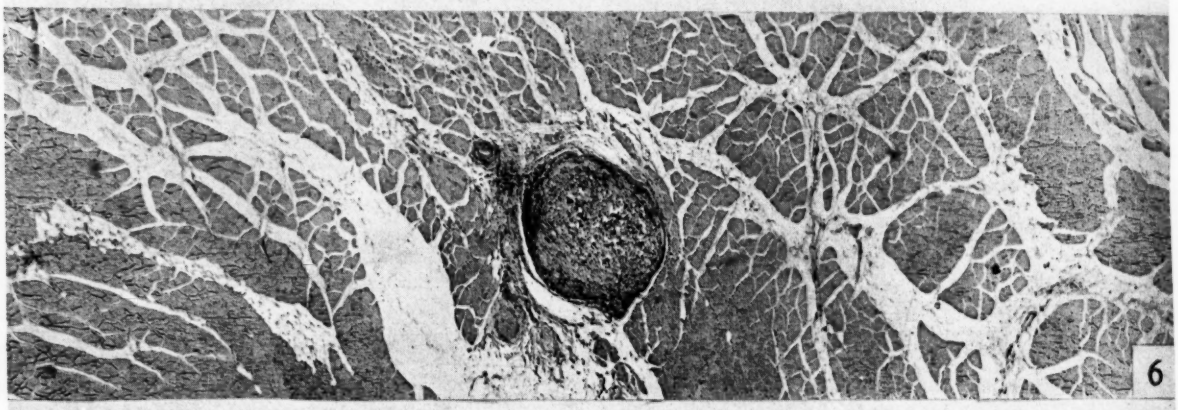
Fig. 1.—Normal skin of mouse showing thickness of epithelium, regular distribution of hair follicles, and thickness of dermis. Mag. × 50.

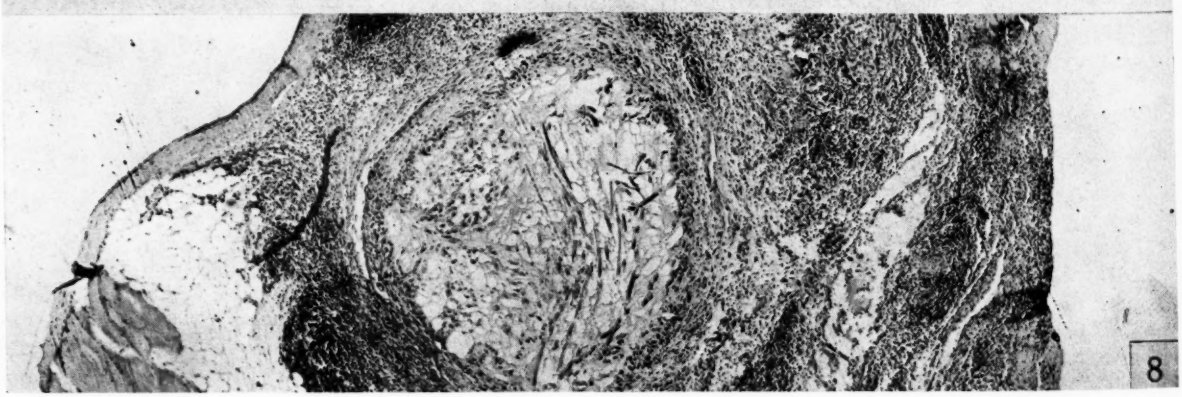
Fig. 2.—Skin of mouse 60 days after treatment with methylcholanthrene. Compare with Fig. 1. Epithelium is thicker, hair follicles have disappeared. In some areas there are regular downgrowths of epithelium, usually in relation to keratin cysts or pre-existing hair follicles. Dermis is many times thicker than normal, and even at this low power more cells and fewer collagen fibers can be seen than in the untreated specimen. Note that smooth muscle is of about the same size and thickness as in Fig. 1. Mag. × 50.

Fig. 3.—High power photomicrograph of collagen fibers of normal mouse dermis. Rete peg at top. Note heavy, wavy bundles of collagen with only an occasional mononuclear cell and fibroblast between them. Compare with Fig. 4. Mag. × 850.

Fig. 4.—High power photomicrograph of dermis of mouse after treatment for 60 days with methylcholanthrene. Epithelium at top. Change in character of epithelial cells. Collagen of dermis no longer arranged in heavy, thick bundles, but cribriform. Compare with Fig. 3. Remaining strands irregular, sparse, and everywhere infiltrated with fibroblasts and an occasional lymphocyte. No leukocytes present. Mag. × 850.







FIGS. 5-8

cinogenic threads could be distinguished on gross examination from those of the noncarcinogenic threads. The cells extended out in streams into the adjoining muscle. After the first 10 days nearly all were of the mononuclear variety, round fibroblasts and lymphocytes being present in about equal numbers (Fig. 10). Multinucleated giant cells were rarely seen, and the occasional ones found contained vacuoles. In the later periods no such cells could be seen, indicating that those observed earlier had been destroyed.

After 2 or 3 months the mononuclear cells in the halo began to increase in number and they came to occupy a considerable portion of the surrounding muscle, but still remained at a distance from the thread. It was among these cells that the sarcoma developed (Fig. 11).

From the start the fibers of the carcinogenic thread were widely separated, and they never became individually surrounded with reticulin like those of the control thread. Some rudimentary fibrils were immediately deposited about the thread on its insertion, but at a comparable time they did not take Van Gieson's stain as did those about the control thread; when some of them did take the red of this stain some months later, the red material was gathered into heavy, irregular thick clumps, and at the same time other fibrils remained fine, were sparse, and still stained yellow (Fig. 12). Eventually all these fibrils also were destroyed.

The morphology of the cells in the surrounding zone began to change before they could be definitely identified with certainty as typical of sarcoma. From the second week on, for example, considerable space always existed between them (Fig. 10). At 90 days many were large fibroblasts that had not assumed the spindle shape. Reticulin fibrils did not appear between them even after so long a period of time. At 150 days nearly all were large, round fibroblasts, and many now varied in size and contained mitotic figures. Thereafter they grew in solid sheets and invaded surrounding structures.

The failure of the tissues to encapsulate properly the carcinogenic thread was expressed also by its position in the tissues. Two or 3 weeks after insertion the carcinogenic thread was always found clumped into a small ball, while the control thread remained in the position of insertion.

In the rats and mice the yield of sarcomas about these threads was high, 92 per cent. They developed earlier in the mouse than in the rat. No tumors were obtained in the guinea pigs.

In summary, then, the following events took place in the evolution of a sarcoma about the thread containing a carcinogen. Muscle about the thread was destroyed by the carcinogen and this area of destruction increased with the passage of time. All the usual cells of exudation appeared, only to be destroyed between the fibers of the thread and in close proximity to it. At a distance from the thread, however, mononuclear cells and fibroblasts persisted, and after an interval of 2 or 3 months began to increase in number, especially the fibroblasts. Reticulin was not deposited between the fibers of the thread, or between the cells at a distance from it. The sarcoma developed in the surrounding cells and not between the fibers of the thread.

Tissue Reactions When Tumors Failed to Develop about Carcinogenic Threads

Tumors failed to develop in some of the rats and mice because the thread, placed in the anterior abdominal wall, moved into the peritoneal cavity subsequent to implantation and hence the carcinogen was not resident in either location for a sufficient length of time to produce a tumor.

When the thread remained where it was inserted, but a tumor failed to develop, the following reaction was observed in the tissues of rats and mice. After a period of about 90 days the wandering cells gradually became more compact about the thread (Fig. 13). The fibroblasts became spindle-shaped, and then reticulin was deposited to form a thick capsule about the thread. Mononuclear cells beyond the capsule slowly disappeared. Finally fibroblasts appeared between the fibers of the thread and reticulin was deposited.

In the guinea pig an entirely different reaction was observed to the carcinogenic thread—a reaction that

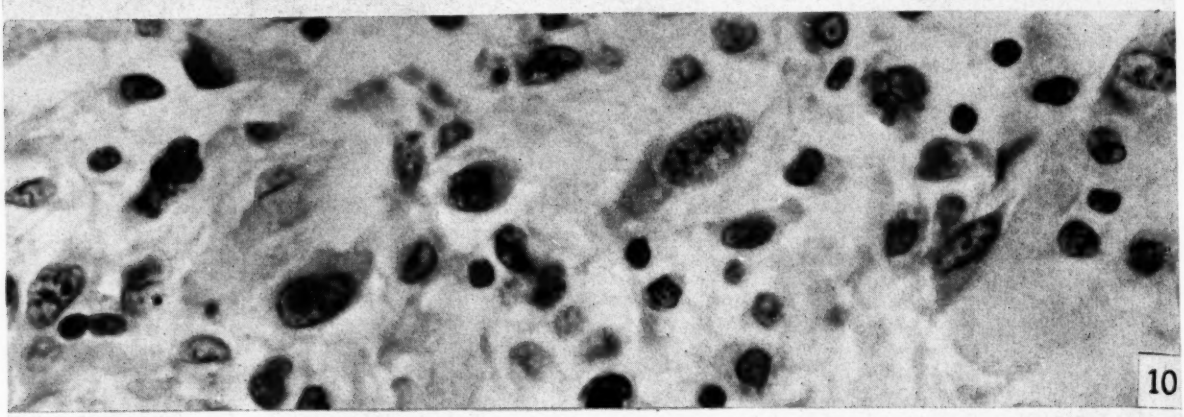
DESCRIPTION OF FIGURES 5 TO 8

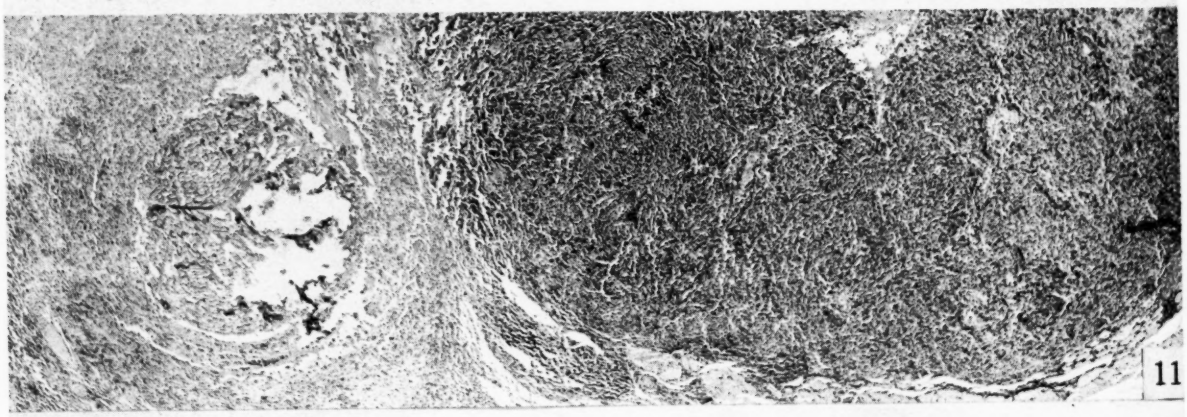
Fig. 5.—Defect on left. Edge of regenerating epithelium just to left of center. Old stratified, keratinized epithelium on right becomes a single layer as the cells move out by ameboid motion. Mag. × 400.

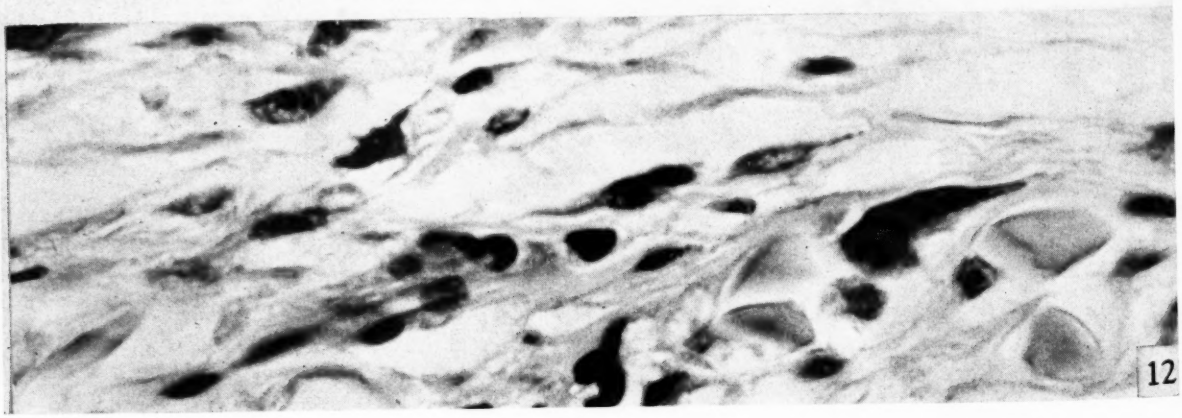
Fig. 6.—60 day 1,2-benzanthracene thread. Process of encapsulation completed. Mag. × 31.

Fig. 7.—Cells between fibers of control silk thread impregnated with 1,2-benzanthracene, 38 days after embedding in muscle of rat. Focus is on the cells, hence thread somewhat indistinct. It may be seen, however, in the lower left hand corner. Numerous fibroblasts and large multinuclear giant cells present. Compare with absence of cells between fibers of methylcholanthrene thread (Fig. 9). Both are of the same magnification. Mag. × 860.

Fig. 8.—Extensive zone of destruction and cellular infiltration around thread impregnated with methylcholanthrene. Embedded in muscle of rat 158 days before. Note absence of cells between fibers of thread. Compare with Fig. 6. Mag. × 31.







FIGS. 9-12

seemed to explain the relative immunity of the species to the action of the carcinogen. Leukocytes immediately infiltrated between the fibers of the thread in spite of the presence of methylcholanthrene (Fig. 14). Moreover, most of them stained normally and continued to remain in this location for a long period of time; only a few showed evidence of being destroyed. Hence the leukocytes were able to phagocytize the carcinogen, and as a result a reticulin capsule was usually well formed by the 40th day (Fig. 15). This capsule consisted of long, regular strands of reticulin fibrils circularly arranged between spindle-shaped fibroblasts. Its central ring stained red, while its periphery stained yellow. The cells of the exudate did not spread beyond the capsule, or if they were present beyond it they were found contracted into a compact zone later in the manner typical of the slower process of encapsulation (Fig. 16). Normal foreign body giant cells were also seen. After 2 or 3 months fibroblasts finally appeared between the fibers of the thread, and a reticulin network was deposited.

Correlation of Tissue Reactions

Certain changes caused by the carcinogen in the skin were so similar to those occurring about the embedded carcinogen that they should be pointed out.

In the skin, cells were initially destroyed by the carcinogen. Some in the germinal layer survived, however, and proliferated to restore the original structure. The regenerated cells were not destroyed again by subsequent applications of the carcinogen, but continued to exist in a hyperplastic, hypertrophied state under the influence of repeated injury. The collagen fibers of the dermis were also injured by the carcinogen and their regeneration did not take place properly. All the large collagen fibers were destroyed, and only a sparse and undifferentiated reticulin network, containing many mononuclear cells, was regenerated. This new network, which was arranged in a thicker layer than the original collagen fibers, failed to differentiate with the passage of time but instead, like the epithelial cells, continued to show evidence of injury. Large collagen fibers did not re-form under the action of the carcinogen, presumably destroyed in the same manner as the original collagen fibrils. Finally, the hyperplastic epithelial cells began to invade the underlying and changed derma and the cells grew in sheets.

Similarly, cells were initially destroyed about the buried thread containing the carcinogen. Leukocytes, mononuclear cells, multinuclear giant cells, and fibroblasts were destroyed both within and adjacent to the thread. Again, however, certain cells—fibroblasts and mononuclear cells—survived, chiefly at a distance from the focus of destruction. Moreover, newly formed reticulin was destroyed and hence, as in the derma, it was not properly deposited either about or within the thread. While the intercellular substance became more disorganized the fibroblasts remained numerous, large, and round, and finally they began to proliferate and grow in sheets and to invade between surrounding layers of tissue.

DISCUSSION

The high yield of tumors obtained in susceptible animals suggests that this method of packing a carcinogen into a thread is a valuable one for studying the production of malignant tumors. A chemical, for example, can be suspected within a short period of time of being a carcinogen by observation of the following criteria: 3 weeks after implantation in a susceptible animal there should be destruction of cells within the interstices of the thread, coupled with a collection of mononuclear cells spreading outward into the surrounding tissues without deposition between them of reticulin, which stains red with Van Gieson's stain. Further, the method presents an opportunity of removing the thread at any time to study the changes leading to the malignant transformation.

The change produced by the carcinogen is a local phenomenon at first, for another thread impregnated with a somewhat similar compound became encapsulated in other muscles in the same animal. It is quite remarkable, therefore, that a change is insti-

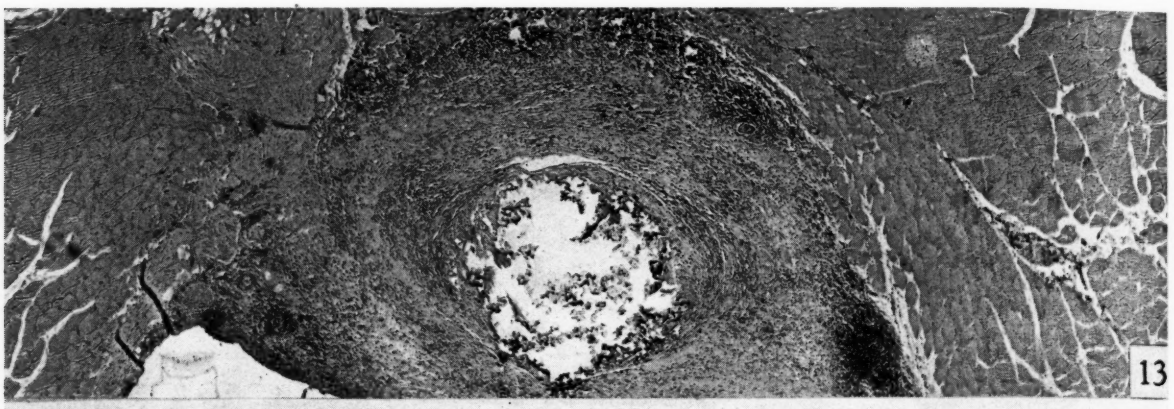
DESCRIPTION OF FIGURES 9 TO 12

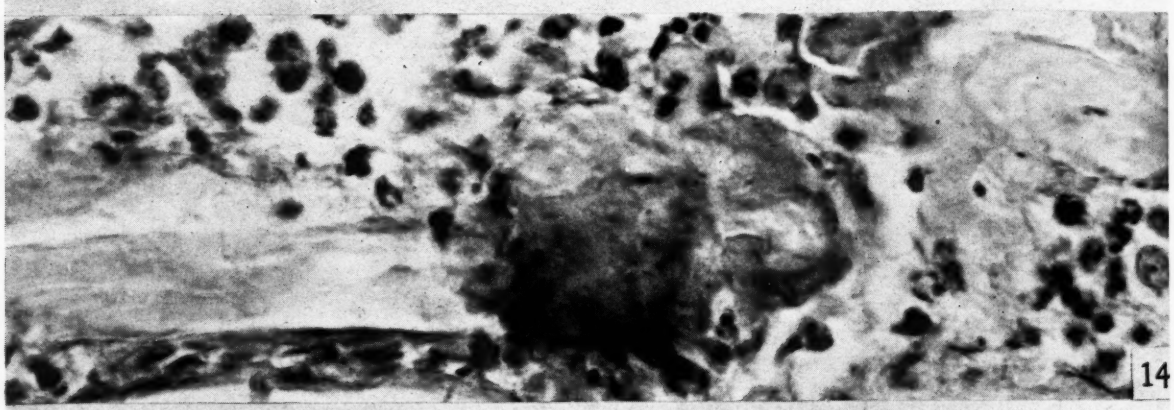
Fig. 9.—Silk thread containing methylcholanthrene embedded in muscle of rat for 158 days. Fibers of thread widely separated. Only cells seen are occasional shadow forms. By contrast, spaces between control thread were completely filled with cells at 38 days (Fig. 7). Mag. × 860.

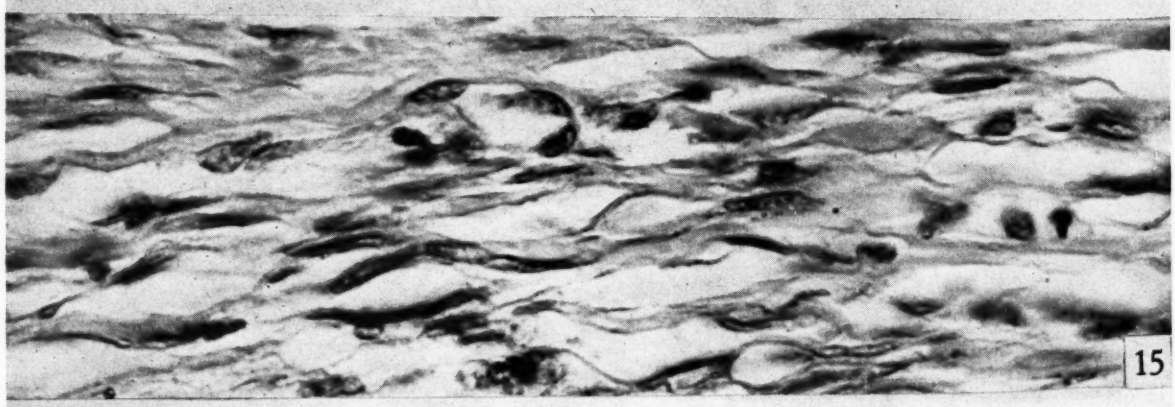
Fig. 10.—Cells in zone beyond imperfect capsule formed about methylcholanthrene thread inserted in muscle of rat 90 days before. Cells are all of mononuclear variety. Many may be identified as lymphocytes and fibroblasts, while others exhibit variations in size and shape and some nuclei have mitotic figures. No regular strands of collagen or reticulin deposited between them. It is in this zone that tumor appeared some time later. Mag. × 860.

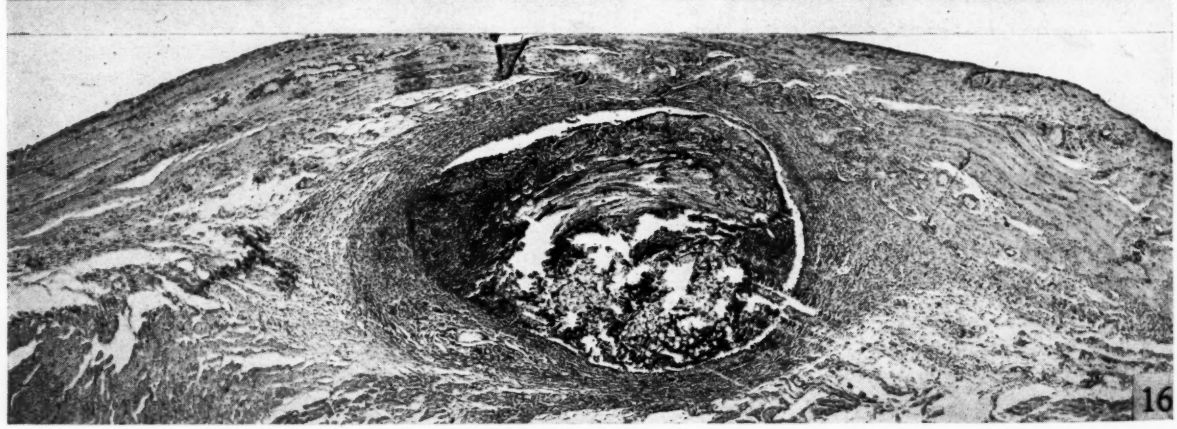
Fig. 11.—Sarcoma right, thread left. Thread embedded in muscle of rat for 240 days. Destruction and cellular infiltration all about thread. No capsule has formed about it. Mag. × 31.

Fig. 12.—Attempted capsule formation about silk thread containing methylcholanthrene embedded in muscle of rat 90 days before. Cross sections of silk fibers can be seen at right. Some rudimentary fibers of reticulin near thread. Compare with Fig. 15. Both have the same magnification. Mag. × 860.









FIGS. 13-16

gated allowing certain cells to proliferate to such an extent that their activities destroy the host. An attempt will be made to interpret these changes that finally lead to malignant proliferation, calling attention especially to those occurring in the connective tissues.

The ordinary guinea pig is resistant to the action of methylcholanthrene because its leukocytes and giant cells are not destroyed by the chemical. Hence, being phagocytized, methylcholanthrene does not remain long enough to produce the malignant change. Shimkin (14) has employed a strain of guinea pigs, however, that is unusually susceptible to the action of methylcholanthrene, and this strain exhibits also peculiarities of fundamental anatomical structures such as polydactylism. Benzpyrene, with which Haagensen (4) produced sarcomas in guinea pigs, was not tested in these experiments.

Failure of the carcinogen to produce tumors in some of the susceptible animals can probably be explained by exhaustion of the chemical from the thread; in other words, a destructive focus did not continue long enough to bring about the malignant change. The escape of the thread from the abdominal wall to the peritoneal cavity, with the failure of a tumor to appear, shows that the focus of destruction must be active for a considerable period of time. The recent work of Simpson and Cramer (17), demonstrating that methylcholanthrene is chemically bound to sebum for a period of days, illustrates the point that in the skin, also, the chemical continues to be present for a longer period of time than if the single application simply bathed its surface. As a corollary, the length of time during which the skin is subjected to the action of methylcholanthrene cannot be estimated from the frequency of its application.

No evidence was obtained from these experiments to indicate that the carcinogen continued to stimulate the division rate of fibroblasts or epithelial cells until a malignant tumor developed. Epithelial cells were initially destroyed by the carcinogen and they actually exhibited a slower rate of division than those in the controls, injured in another manner, when they began to proliferate again after a lag period of 7 to 10 days. The cells did not become quiescent again, however, as is usual after a single injury, but remained hyperplastic and hypertrophied as they do in some forms of continuing injury. They remained in this state until a new mass of cells, typical of the epithelioma, was produced 10 to 15 weeks later, when they became invasive. Reller and Cooper (12) also have observed that from the third to the ninth week after the first application of a carcinogen to the skin, the number of mitoses decreased sharply from the number found at the end of the second week, though they were still more numerous than in untreated epidermis. Unfortunately, these investigators did not compare the frequency of mitoses in epidermis made hyperplastic by another form of injury.

Martin and Ruth Silberberg (15) have reported on the effect of benzpyrene upon the healing of wounds in skin that had been treated for some time before the injury was inflicted. They prepared the skin of mice by applying 0.3 per cent benzpyrene in benzene 3 times weekly for 2 weeks, 1, 2, and 3 months before a 3 mm. incision was made. The controls were treated with benzene alone. The wounds were examined microscopically after 3, 5, 8, and 10 days for the number of mitotic figures in the new and old epithelium and for the length of extension of new epithelium over the defect. Benzene hastened the closure of the wound and produced more mitotic figures both in the old and new epithelium. Benzpyrene affected the closure and the number of mitotic figures similarly to benzene, except after 3 months, when benzpyrene was already producing papillomas; then the number of mitotic figures was only increased comparably to the wound treated with benzene, but extension of new epithelium across the defect was inhibited and closure of the wound definitely delayed. At the same time the epithelium tended to extend downward into the dermis rather than laterally. To explain this phenomenon, the Silberbergs called attention to the possibility that the base of the wound might have been injured by the benzpyrene in the manner described by Orr (8).

DESCRIPTION OF FIGURES 13 TO 16

FIG. 13.—Methylcholanthrene thread in muscle of rat for 240 days. No tumor has appeared. Area of destruction circumscribed. Cells, largely lymphocytes, oriented about thread, though some are still beyond a thick capsule. New blood vessels have appeared at the border of healthy muscle. Mag. × 31.

FIG. 14.—Cells between fibers of methylcholanthrene thread embedded for 51 days in resistant guinea pig. Although some shadow forms are present, most of the cells take hematoxylin well. Many are leukocytes. This infiltration is in definite contrast to sparsity of cells between fibers of carcinogenic thread in nonresistant animal. Solid material in the crater is methylcholanthrene. Mag. × 860.

FIG. 15.—Collagen fibers in capsule about methylcholanthrene thread in resistant guinea pig after 51 days. Note even, regular, thick, deposition. Compare with capsule formation at 90 days about methylcholanthrene thread in rat (Fig. 12). Mag. × 860.

FIG. 16.—Encapsulation of methylcholanthrene thread in muscle of resistant guinea pig. Thread inserted 51 days before. Many cells between fibers of thread. Capsule thick and regular in depth. No area of destruction, but some cellular infiltration, extends beyond capsule on left. Mag. × 31.

In other words their results, like ours, did not show stimulation of the proliferation of epithelial cells over and above that caused by injury.

In the same way, fibroblasts did not divide at an increasing rate about the carcinogenic thread in the present experiments; in fact, more fibroblasts and mononuclear cells seemed to be present about the thread 3 weeks after implantation than in the next 40 or 50 days. An unusual number was always present, of course, in response to the continuing injury. However, after a length of time equivalent to that required for the epithelial cells, a new mass of fibroblasts appeared and they grew in sheets and thereafter invaded the surrounding tissues.

The phenomenon to explain is the new mass of cells—fibroblasts and epithelial cells—that appeared 10 to 15 weeks after exposure to the carcinogen. A possible explanation is that stimulation of the rate of proliferation of these cells occurred late from a secondary chemical change instituted by the carcinogen. Cowdry and his group (1) believe that a substance called proliferin is produced in the cells by the action of the carcinogen. Although they have not described just how it works one assumes that it continues to accumulate, thereby increasing the rate of cell division until a tumor forms. This explanation, however, does not allow for the possibility that the effectiveness of the carcinogen in a thread, at least, must be progressively diminishing, and does not tell why the process of fibroplasia did not evolve normally in the area of injury. Nor would it account for the invasiveness of the new mass of cells.

No real evidence has been produced to support the thesis that an increased rate of division will cause cells to invade normal surrounding structures. For example, those of epithelium do not become invasive in hyperplasia of the female breast during lactation; or during wound healing, when there is an increased rate of cellular division. In the breast, surrounding reticulin expands without previous fibroplasia; in the wound, fibroplasia precedes epithelial proliferation. Hence the theory of an extraordinary stimulation of cells continuing until the malignant change occurs should be abandoned.

On the other hand the late appearance of the new mass of cells, and their invasiveness, could be explained by the type of injury that the carcinogen caused in tissues and the distortion produced by it in the regenerative patterns that followed. Two types of cells survived the initial injury—the fibroblast and the epithelial cell. These responded to the continuing insults resulting from the continuing presence of the carcinogen by becoming hyperplastic and hypertrophied, but did not extend at once beyond their usual situation because the collagenous fibers and the reticulin network were still present.

The collagenous fibers and the reticulin network were initially destroyed by the carcinogen, yet the larger fibers remained *in situ* and were only gradually absorbed; in the rat, absorption of the old collagenous fibers took a month or more whereas it occurred more rapidly in the mouse. Fibroblasts and mononuclear cells that were uninjured by the carcinogen appeared between them, and while some fine fibrils were elaborated any attempts to produce new differentiated reticulin and collagen fibers were blocked by the action of the carcinogen. Ultimately a condition was reached where regeneration and differentiation of the connective tissues failed, old barriers were no longer present, and the hyperplastic and hypertrophied fibroblasts and epithelial cells grew in sheets amongst the collagen fibers (Figs. 3 and 4). They became naked cells, so to speak, which, already possessing ameboid motion, could travel into locations where the impetus to reestablish connective tissue boundaries no longer existed. Here they would grow in metastatic foci.

There is ample evidence that adult naked fibroblasts and epithelial cells would continue to proliferate if they were free of intercellular substance. Simms (16) has demonstrated that if the vitality of cells is preserved while the ground substance is loosened, as for example by trypsin, adult cells will grow in tissue cultures; moreover, they have a limitless capacity for proliferation, as culturing and subculturing have shown. The cells of transplantable tumors are also naked cells. They have a very sparse and fine reticulum, which takes silver, but not the red of Van Gieson's stain except around blood vessels.

The importance of the destruction of connective tissue by the carcinogen is emphasized by the ubiquity of reticulin and collagenous fibers. In the adult forms of animal life reticulin and collagen normally surround most cells regardless of their proliferative activities. Epithelial cells are limited by basement membranes such as the derma, or they lie in reticulin; in the same way, fibroblasts lie dormant between bundles of collagen except for certain nests of them near blood vessels, or differentiate from wandering mononuclear cells brought to the site of the injury by the blood stream.

Distortion of the size and shape of cells, which characterize the malignant growth, can be interpreted as loss of the proper boundaries between cells, as well as evidence of nuclear disturbance. Indeed, the Lewises (7) have verified once more the opinion that atypical mitoses do not characterize the malignant change; numerous mitoses are found here merely as they are found in all growing tissues.

Destruction of the collagenous fibers of the derma under the influence of carcinogenic agents has been reported ever since tar was first used to produce epithelial neoplasms (20), but usually was dismissed

as inflammatory until Wolbach (21) and Orr (8) suggested that it might have another significance.

Destruction of the derma alone would not produce the malignant transformation, of course, because its destruction follows the application of many other chemicals that do not produce tumors. The alkalies and the salicylates readily destroy derma, and it becomes replaced with a fine reticulin; however, epidermis is likewise destroyed, and does not regenerate until after cessation of the application of the chemical.[2] Other substances, including most of the hydrocarbons and the sulfhydryl compounds (11) cause hypertrophy and hyperplasia of epithelial cells, yet leave the collagen of the derma unharmed; or, if harmed, capable of progressively changing until an adequate connective tissue barrier is re-formed. In wounds of the skin, for example, fine reticulin fibrils appear and the epithelium becomes hyperplastic, but these continue to hypertrophy and to differentiate. The carcinogens are unique because they destroy the derma and render it incapable of regeneration, while leaving epithelial cells capable of proliferation. The buried carcinogen is also unique because at first it is not phagocytized; and again, it destroys collagen and reticulin fibers, leaving fibroblasts capable of proliferating but incapable of re-forming reticulin and collagenous fibers.

Recent work by Earle and his group (3) on culturing fibroblasts *in vitro* in the presence of a carcinogen helps to strengthen the hypothesis herein expressed: That the malignant transformation does not result from an increased rate of cell division alone, but from a fundamental change in ground substance plus hyperplasia. These investigators could not demonstrate that the rate of mitotic division was increased after long exposure to methylcholanthrene, although the cells became malignant. At first their rate of growth was depressed in the presence of the carcinogen; no widespread degeneration or disintegration took place, but there was a definite retardation in the frequency of mitosis. After a time the slender terminal processes of the cells associated with assumption of the spindle shape and deposition of reticulin did not form. Then the cells developed increased ameboid motion, finally became round, and tended to form loose sheets and ribbons. After these changes had occurred they were injected into mice, and grew as sarcomas.

Contradiction to the conclusions of these experiments—that the carcinogen destroys connective tissues and prevents their proper regeneration—will necessarily stem from contrasting this possibility with the behavior of malignant tumors in general.

[2] Through the courtesy of Dr. Gray and Dr. Cairncross, of the Arthur D. Little Company, of Boston, I have had the privilege of examining microscopic slides of pig skins treated for 2 or 3 weeks with many different chemicals.

The enormous amount of connective tissue about a scirrhous carcinoma, the fact that wounds heal in patients with cancer, and that they heal even within a cancer, will call attention to the evidence that there is no failure of fibroplasia in the patient with cancer. Consequently it will be argued that malignant tumors cannot originate in this manner. However, there is no reason why the process of cicatrization should be changed in the patient with a spontaneous cancer any more than it was in the rats or mice used in these experiments, which developed tumors from local contact with the carcinogen. The escape of hyperplastic cells need occur in only one location without the necessary stimulus to initiate subsequent fibroplasia.

No hasty conclusion should be drawn that this investigation revives the theory of Thiersch (19); namely, that a malignant epithelial tumor originates because of a disturbance of growth equilibrium between connective tissues and epithelium; because the growth energy of the connective tissue, including that of the fibroblasts, failed.

Also, until additional proof is obtained, no attempt should be made to explain the origin of spontaneous malignant tumors from the evidence herein presented. It is hoped that this paper will call attention to a phase of oncology that has been too much neglected, while study was confined to the behavior of cells. In view of the newer knowledge of the fine structure of collagen and reticulin, as revealed through the electron microscope by Schmitt and his co-workers (13), it should be possible to investigate the changes in the collagenous fibers and the reticulin that occur in spontaneous tumors.

CONCLUSIONS

1. Collagen fibers are destroyed in the dermis as the result of repeated applications of a carcinogen, whereas epithelial cells survive and remain hyperplastic.

2. The fibers regenerated in the dermis under the influence of the carcinogen are sparse and do not differentiate. Many mononuclear cells are present between them.

3. The rate of epithelial proliferation in the presence of the carcinogen is not increased above the rate occasioned by another form of injury.

4. Muscle is destroyed about the buried carcinogen, together with the phagocytic cells that might remove it from the site.

5. Fibroplasia is notably altered by the buried carcinogen. Reticulin fibrils are not produced between the fibroblasts; or if they are produced they are destroyed again, as in the dermis. The fibrils do not differentiate. On the other hand, fibroblasts survive the destruction, particularly at a distance from the focus.

6. In both anatomical locations the carcinogen leaves the cells—fibroblasts and epithelial cells—free to proliferate because of the lack of properly organized intercellular substance, and hyperplastic because of injury.

7. It is suggested that the late appearance of the tumor is due to the time required for the damaged collagen fibers to be absorbed and for the re-formation of new connective tissue barriers to fail.

8. No justification can be found for the theory that the carcinogen stimulates the cells directly to the formation of a tumor.

9. The usual guinea pig is resistant to carcinogens because leukocytes and giant cells are not destroyed by the chemical; hence the chemical is phagocytized.

10. The use of a thread impregnated with a carcinogen is recommended as a means of studying the production of tumors. It might be useful, also, to determine whether a chemical is a carcinogen.

11. A plea is made for a more intense study of the significance of connective tissue in neoplasia.

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The Etiology of Fowl Tumors

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The purpose of this communication is to bring up to date an experimental study of 55 chemically induced fowl sarcomas, first described some 12 years ago (16-18), and to compare these results with others obtained in parallel experiments with 3 infective fowl sarcomas transmissible by cell-free material. The bearing of these results on the theoretical problem of the etiology of cancer is discussed.

Since 1935, when a comprehensive account of the transplantable chemically induced sarcomas, G.R.C.H. 1 to 14, was published, one more transplantable tumor strain, derived from a dibenzanthracene sarcoma (G.R.C.H. 15), has been under investigation, and is in the 31st generation of serial transplantation at the time of writing. The remaining tumors of the G.R.C.H. series have all died out; they were transplantable with very variable success, and none could be transmitted by cell-free material (Table I).

Attempts were made to transmit 28 of the remaining 40 chemically induced tumors, but without success. Sarcomas G.R.C.H. 2 and 12 are omitted from Table I and from further discussion, because they retrogressed during the first serial passage without having been histologically confirmed. The 13 transplantable G.R.C.H. sarcomas were in all demonstrable respects similar to transplantable chemically induced sarcomas of mammals.

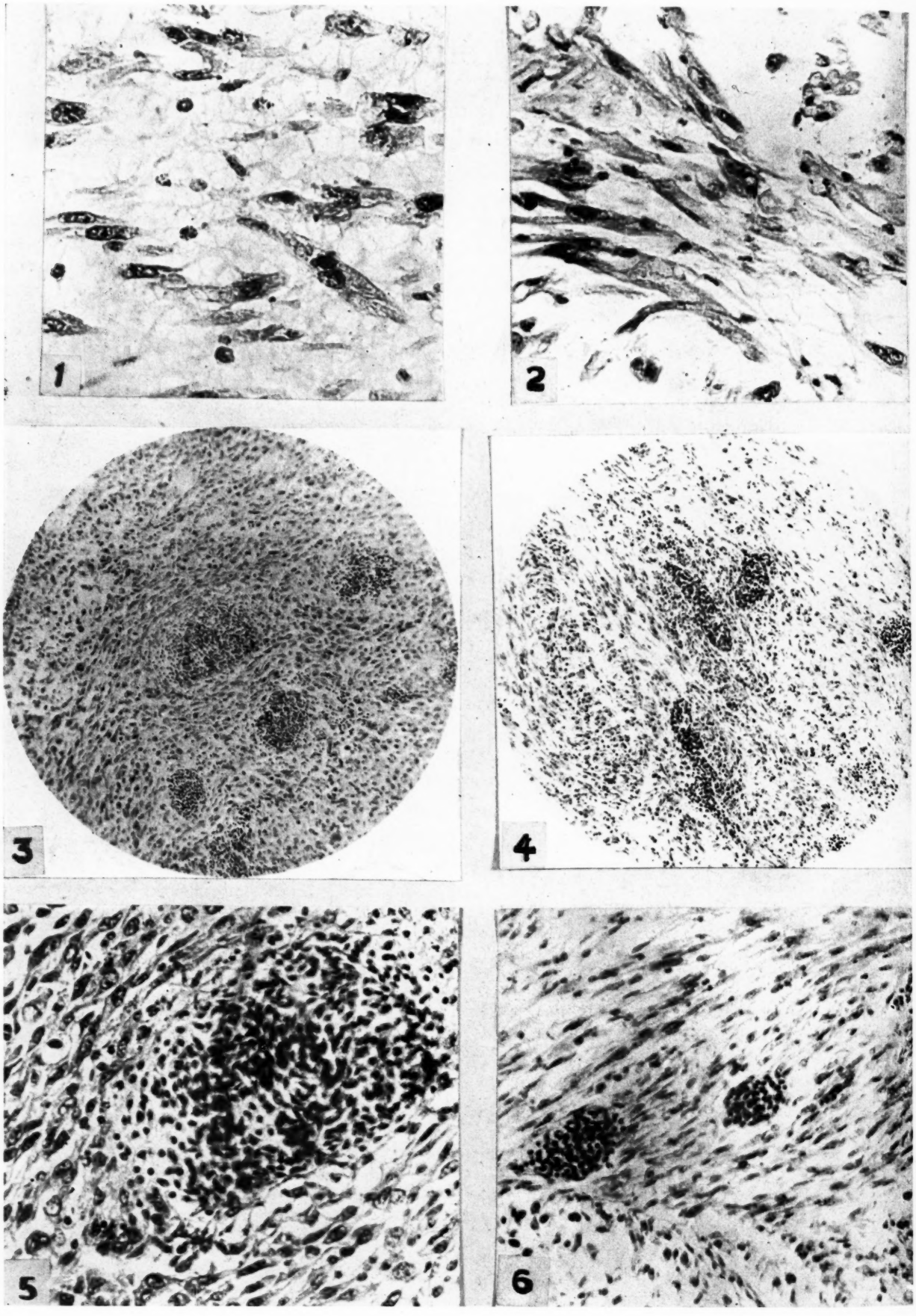
TABLE I

Tumor		Inducing carcinogen	Original breed	Host breed	Serial passages	Successful inoculations			Histology
						Cells +	Cells ±	Cells −	
G. R. C. H.	1	1, 2, 5, 6-Dibenzanthracene (D-B.)	BPR[1]	BPR	2	3/24	1/2	0/7	Whorled spindle cell sarcoma
"	3	Tar	BPR	BPR & RIR[2]	6	47/79	3/22	0/22	Spindle cell sarcoma, sometimes anaplastic
"	4	"	BPR	BPR	3	4/6	—	—	Spindle cell sarcoma
"	5	"	BPR	BPR	7	18/42	2/13	0/22	"
"	6	D-B.	BPR	BPR	2	5/13	0/13	—	"
"	7	Tar	BPR	BPR	2	6/14	—	—	"
"	8	"	BPR	BPR	1	2/3	—	—	"
"	9	"	BPR	BPR	2	1/4	—	0/12	"
"	10	"	BPR	BPR	2	1/5	0/2	0/5	"
"	11	D-B.	BPR	BPR	2	1/3	—	0/4	"
"	13	Tar	BPR	B.L.[3]	23	109/384	4/124	0/56	Spindle cell sarcoma (degenerate tumor sometimes resembles Rous 1)
"	14	"	BPR	BPR	5	11/35	2/15	0/35	Spindle cell or anaplastic sarcoma, sometimes endothelioma-like
"	15	D-B.	B. L.	B. L. & W. L.[4]	31	364/461	98/393	0/60	Spindle cell sarcoma

[1] BPR = Barred Plymouth Rock (market bought).
[2] RIR = Rhode Island Red (market bought).
[3] B. L. = Brown Leghorn (Edinburgh strain).
[4] W. L. = White Leghorn (Reaseheath strain).

Macroscopic Appearances

Dissection of tumors derived from all the types of inoculum employed suggests strongly that in the case of the G.R.C.H. series growth is always from the surviving cells, which develop into tumors only at sites where they have established a connective tissue stroma. Tumors grown from single grafts are roughly spherical in their early stages, as would be expected, and where multiple grafts have been inoculated multiple spherical tumors develop. Successful inoculations of suspensions of these "nonfiltrable" tumors show evidence of multicentric origin, and in macroscopic sections of such tumors a peculiar mosaic appearance can often be seen (Fig. 12). If such tumors



FIGS. 1-6

are exposed as soon as they are clinically detectable many little individual tumors can often be demonstrated, growing independently of one another. At a later stage of development, they tend to crowd each other, giving the mosaic appearance, and they may finally become confluent and lose this characteristic appearance. In proportion as the technical procedure reduces the number of surviving cells, so the number of multiple points of origin in successful inoculations is reduced. In contrast with these appearances, the inoculation of "filtrable" suspensions usually gives rise to diffusely growing sarcomas, which rapidly undergo myxomatous degeneration except at their growing periphery. Such tumors are also multicentric in origin but, as they are derived from infected cells of the new host, they tend to become confluent early in their development, unlike transplanted "nonfiltrable" tumors, which always excite some degree of encapsulating reaction on the part of the host. Occasionally, however, multiple discrete tumors develop along the track of the inoculating needle, particularly where this has traversed fascial planes, suggesting that the injured tissues at these sites provide the most susceptible cells for the action of the virus.

Histological Differentiation

The G.R.C.H. sarcomas conformed to the general plan of compact, whorled, spindle cell sarcoma though considerable variations in appearance could frequently be seen in different parts, depending partly on such secondary effects as degeneration, edema, and bacterial infection. Such appearances sometimes resembled histologically the myxomatous degeneration that is a regular feature of the three virus sarcomas, Rous 1, Fujinami, and McIntosh 5, but the resemblance is only superficial (Figs. 1 to 11). Each of the G.R.C.H. sarcomas remained histologically true to type in subsequent serial passages, and G.R.C.H. 3, 5, 13 (Fig. 1), 14, and 15 (Fig. 11) could usually be distinguished by slight but consistent differences. Thus G.R.C.H. 3 (Fig. 7) and 14 showed a striking perivascular arrangement that gave them an angioendotheliomatous appearance in some sections, particularly when cut across the main axis of the cells, although the bulk of the tumor might be composed of fleshy, spindle cell sarcoma (Fig. 8). By suitable selection of microscopic fields it would be possible to make a case for or against the similarity of these tumors to one another, or to virus tumors, but by surveying many sections it is possible to form a composite mental picture of each type that is sufficiently characteristic for differential diagnosis.

A recent analysis of about 5,000 sections from 1,017 consecutive cases of fowl sarcoma or suspected tumor, without reference to their origin, showed (Table II) that the "filtrable" virus tumors could be distinguished

Table II: Distribution of Error in 1,041* Consecutive Histological Examinations of Fowl Tumors or Suspected Tumors

Lesion	No. of examinations	Non-malignant	Diagnosis				
			Spont. T.	G. R. C. H.	McI. 5	Fuj.	Rous
Nonmalignant	512	512 (100%)	—	—	—	—	—
Filtrability not tested:							
Spontaneous tumor	17	—	16 (94%)	1 (6%)	—	—	—
Nonfiltrable:							
Chemically induced sarcomas (G. R. C. H. series)	403	—	—	390 (96.75%)	3 (0.75%)	5 (1.25%)	5 (1.25%)
Filtrable							
McIntosh No. 5	52	—	—	12 (23%)	39 (75%)	1 (2%)	—
Fujinami	41	—	—	5 (12%)	4 (10%)	32 (78%)	—
Rous 1	16	—	—	—	—	—	16 (100%)

* Histological sections from 1,017 fowls were examined (without reference to their history) and were classified in one of the 6 groups above Twenty-four were wrongly classified and were re-examined without reference to the previous diagnosis. The final error was 36/1,041 examinations failure to distinguish filtrable from nonfiltrable tumors, 30/622.

DESCRIPTION OF FIGURES 1 TO 6

All sections stained with hematoxylin and eosin

Fig. 1.—Fowl 138. Tar sarcoma. Original tumor of G.R.C.H. 13 strain. Note superficial resemblance to Rous sarcoma 1. Mag. × 500.

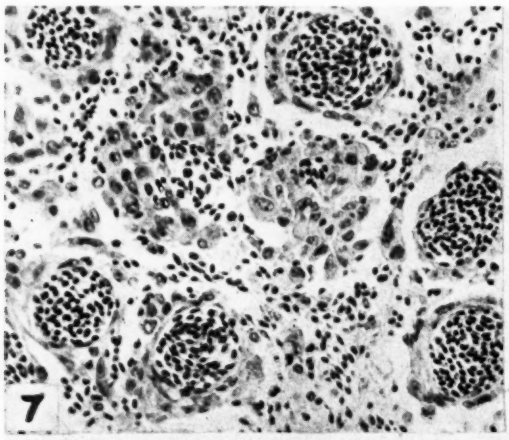
Fig. 2.—Rous sarcoma 1. Mag. × 500.

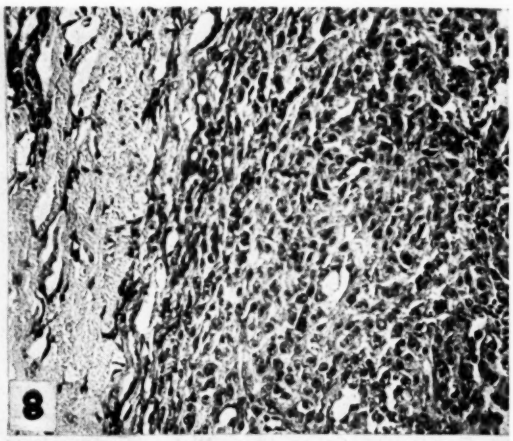
Fig. 3.—G.R.C.H. 14/3. Periphery of tumor. Note large, thin-walled capillaries similar to those seen in Fujinami and McIntosh 5 sarcomas. Mag. × 110.

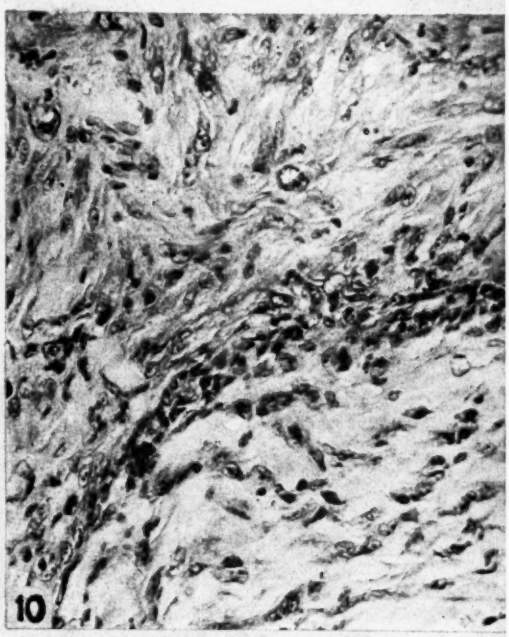
Fig. 4.—Fujinami sarcoma. Mag. × 110.

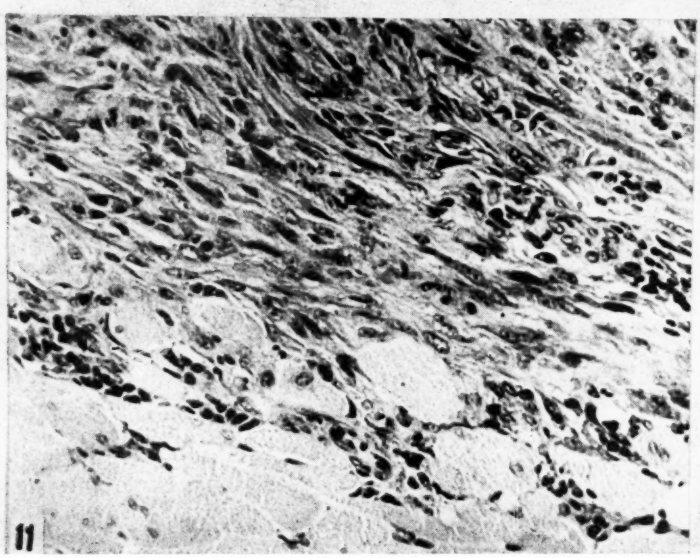
Fig. 5.—Same as Fig. 3. Mag. × 390.

Fig. 6.—Fujinami sarcoma. Mag. × 300.









FIGS. 7-11

from the "nonfiltrable" G.R.C.H. sarcomas with an error of about 5 per cent (19). Obviously the accuracy of diagnosis would vary with different observers, but within the limits of this investigation 15 chemically induced sarcomas were histologically distinguishable from 3 virus sarcomas (Table III).

TABLE III: ANALYSIS OF 24 WRONG DIAGNOSES FROM TABLE II

Path. No.	Correct diagnosis	Histological diagnosis* 1st	2nd	Error
424	Fujinami	G. R. C. H.	G. R. C. H.	2/2
728	"	"	Fujinami	1/2
816	"	"	"	1/2
995	"	McIntosh 5	McIntosh 5	2/2
1061	"	"	"	2/2
1088	"	G. R. C. H.	G. R. C. H.	2/2
2696	McIntosh 5	"	Fujinami	2/2
2714	"	"	McIntosh 5	1/2
2752	"	"	"	1/2
2862	"	"	G. R. C. H.	2/2
2875	"	"	"	2/2
3076	"	"	McIntosh 5	1/2
3105	"	"	G. R. C. H.	2/2
3109	"	"	McIntosh 5	1/2
854	G. R. C. H.	Fujinami	G. R. C. H.	1/2
1058	"	"	Fujinami	2/2
1125	"	"	G. R. C. H.	1/2
1219	"	Rous	"	1/2
1620	"	"	"	1/2
1930	"	"	"	1/2
2205	"	"	"	1/2
2388	"	Fujinami	"	1/2
3104	"	McIntosh 5	McIntosh 5	2/2
3111	"	"	Rous	2/2
2911	Spontaneous	G. R. C. H.	Spontaneous	1/2

* Results under first examination include all the wrong diagnoses from 1017 sections examined by random selection. Under second examination the wrongly diagnosed sections were re-examined without reference to the correct diagnosis.

SUSCEPTIBILITY AND GENETIC RELATIONSHIP

Sarcomas G.R.C.H. 1 to 14 were derived from Barred Plymouth Rock fowls of unknown pedigree, but G.R.C.H. 15 was a dibenzanthracene sarcoma in cockerel K.293 of the Edinburgh Institute of Animal Genetics inbred line of Brown Leghorns.[1] The susceptibility of individual birds to tumor grafts varies considerably, and seems to be unpredictable, except in the case of inbred lines. Thus G.R.C.H. 3 grew equally well in Rhode Island Reds and in Barred Plymouth Rocks; G.R.C.H. 13 grew in Barred Plymouth Rocks and in Brown Leghorns, and was ultimately maintained only in the latter breed until the summer of 1939, when it failed in the 23rd serial passage. G.R.C.H. 14 in its first serial passage grew in 2 out of 4 Brown Leghorns, but "probably cell free" filtrates failed to grow in 2 Barred Plymouth Rock chicks hatched from the original tumor-bearing fowl, and in 4 unrelated Brown Leghorns. This strain was subsequently maintained through 4 serial passages in Brown Leghorns, but died out in the fifth passage, during the summer of 1934. G.R.C.H. 15 is the only tumor in the series that was induced and maintained by serial transplantation in birds of the same inbred strain of Brown Leghorns. Even so, the susceptibility of individual birds to this tumor has shown great variability. This can be correlated to some extent with genetic factors peculiar to several lines within this inbred strain (9). On several occasions attempts were made to transmit G.R.C.H. 15 sarcoma to White Leghorns of the Reaseheath inbred line. In one experiment the tumor grew in 1 out of 9 White Leghorns and in 1 out of 5 Brown Leghorns; but a similar test on another occasion yielded no tumors in 4 White Leghorns, and grew in 5 out of 5 Brown Leghorns (Chart I).

[1] Purchased with a grant from the British Empire Cancer Campaign.

CLASSIFICATION OF EXPERIMENTAL RESULTS

The main purpose of all the transmission experiments with chemically induced fowl tumors was to search for hypothetical causal viruses. The interpretation of results depends on the criteria accepted for cell-free transmission. The only strictly cell-free tumor extracts are those from which it can be asserted that anything of cellular dimensions has been mechanically excluded. In practice, this means filtrates through bacteria-fast filters and supernatant fluid from centrifuged extracts. But several other technics yield extracts free from living cells, as far as can be demonstrated by inoculation and by microscopic examination. In the cell-free category, therefore, it is proposed to include filtrates through bacteria-fast filters, centrifuged supernatant fluid from tumor extracts, desiccates, tumor cells exposed *in vitro* to more than 23,000 r of x-rays or gamma-rays, finely suspended tumor cells treated with 50 per cent glycerine for more than 24 hours, and capillarity filtrates (described below). All these methods have yielded positive results in the case of

DESCRIPTION OF FIGURES 7 TO 11

FIGS. 7 and 8.—G.R.C.H. 3/5. Different fields from same section, fowl 588. Note striking contrast in appearance. Mag. × 233.

FIG. 9.—McIntosh 5. Mag. × 166.

FIG. 10.—McIntosh 5 (No. 2752, Table III). This field closely resembles typical G.R.C.H. sarcomas. Mag. × 233.

FIG. 11.—G.R.C.H. 15/5. Typical of this tumor strain. Note lymphocytes at invading edge of tumor, characteristic of all grafted tumors in G.R.C.H. series. Mag. × 331.

virus tumors. In a previous communication (17), material used for test inoculations was classified under one of three headings: (a) living cells; (b) probably cell-free; and (c) cell-free. Of these categories, only the first and last could be clearly defined. In the light of subsequent experience the second category might better have been subdivided into "probably not cell-free," and "probably cell-free" groups, as the probability of cells surviving the various procedures grouped together in the second category varies considerably; *e.g.*, cells can often be demonstrated in preparations of tumors ground with sand and only lightly centrifuged, whereas this is not true of filtrates through a 1 inch layer of sand covered by 1 inch of packed paper pulp or surgical cotton wool.

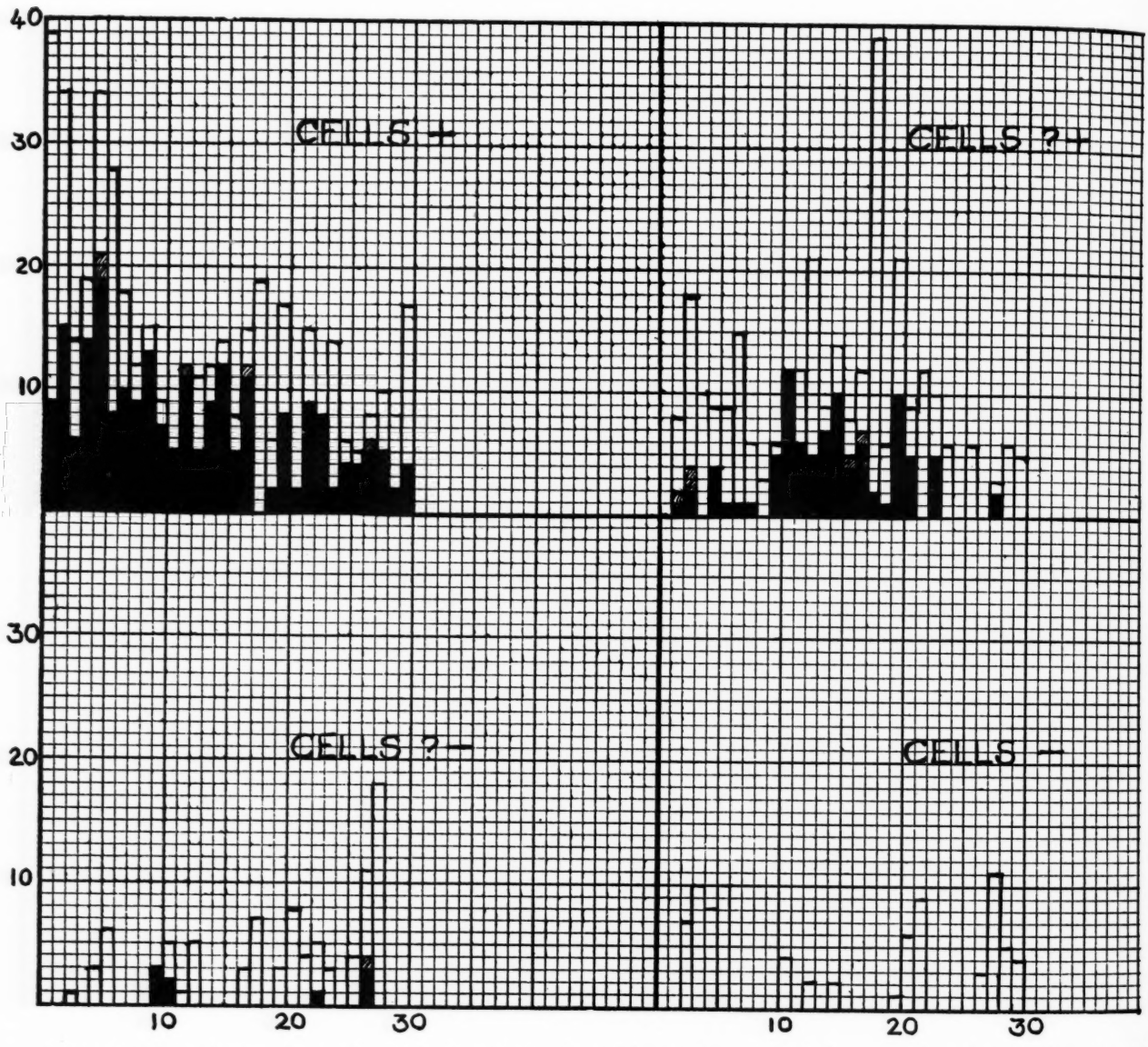


CHART I.—The ordinates give number of fowls used at each serial transplantation; abscissae, number of generation of serial transplantation. Note no absolute time scale is implied (chart does not differentiate between slowly growing and rapidly growing tumors).

Black = progressively growing tumors. Cross-hatched = retrogressed tumors. White = no tumor growth.

SEASONAL FACTORS

Grafts established at the time of the first biopsy on fowl K.293 were maintained through 4 serial transplantations, but died out during the midsummer months of 1940. In this respect the history of this tumor resembles that of the earlier G.R.C.H. sarcomas, in which a seasonal influence of undefined nature was inimical to the transplantation during the summer months (17). A similar effect has been described recently by Murphy and Sturm (12) as influencing their dibenzanthracene sarcomas. However, grafts of G.R.C.H. 15 made at the time of the death of K.293 were successfully maintained through this same period, though at one time only 1 bird bearing a tumor was

available for experiments. The transmissibility of this tumor is summarized in Tables I, III, IV, and Chart I, from which it may be seen that the success of transmission depends not only on the new host, but also on the source and nature of the material. For example, at the 15th serial passage tumor inoculated on 3 separate occasions grew in most of the birds, whereas at the 29th generation the same tumor, tested on 2 occasions, grew in only a third of the birds tested (Chart I). It seems reasonable to suppose that in serial passages where positive results were obtained with material classified in the first 3 categories in Chart I, the best opportunity occurred for the demonstration of a causal virus, if one were present. However, as previously stated, cell-free material failed at every test to reproduce the tumor. The G.R.C.H. 15 sarcoma, therefore, resembles all the other chemically induced tumors studied in these laboratories, as well as the series of chemically induced sarcomas described by Murphy and Sturm (12, 13), who also emphasized the remarkable difference in behavior of such tumors compared with the spontaneous transmissible fowl tumors that have been investigated since 1910 in the laboratories of the Rockefeller Institute and elsewhere.

In contrast with these results are those of McIntosh (10) and McIntosh and Selbie (11, 21), who said that they had established a causal relationship between injections of tar into fowls, and the subsequent recovery from tumors occurring in such birds of viruses capable of initiating sarcomas in fowls. Further reference will be made to this in the discussion that follows, but it should be said at once that 5 of the 10 tumors in the McIntosh series appear to resemble the chemically induced, "nonfiltrable" tumors of fowls described by other workers, while the remaining 5 "filtrable" tumors form a peculiar group in which multiple factors of possible etiological importance have to be considered.

TABLE IV: EFFECT OF MECHANICAL DISINTEGRATION ON G. R. C. H. 15 SARCOMA

Relative vol. of inoculum	Size of tumor					
	a < b	a = b	a > b	a + b −	a − b +	a − b −
a = b	33	4	9	7	2	22
a = 2b	2					
a = 4b	2					
a = 8b		1	1			

a = Tumor ground with sand.
b = " " without sand.

TECHNICAL CONSIDERATIONS

Grinding tumors with sand is the routine preliminary in preparing infective tumors for filtration, and there is ample evidence that their transmissibility is not destroyed by this procedure. In the case of most mammalian tumors, however, mechanical disintegration, if thoroughly carried out, greatly reduces or destroys their transmissibility, which depends on the survival of tumor cells. The method should therefore give circumstantial evidence for or against a causal virus in chemically induced fowl tumors. In these laboratories it is done as follows. Fresh tumor is minced and divided into equal portions, (a) and (b): (a) is ground with sand until of the consistency of thick mucilage and 0.9 per cent saline is added to make a dilution of 1:20; (b) is ground without sand to the same consistency, and saline is added to give a similar dilution of tumor suspension. A small quantity of sterile sand is added to (b), and both suspensions are shaken and then allowed to sediment for 15 minutes. Progressive dilutions of the supernatant, opalescent fluid from (a) and (b) suspensions are injected in equal amounts into opposite pectoral muscles of a series of fowls. The result of test injections in 83 fowls are summarized in Table IV. There is usually an obvious reduction in the size of the tumors resulting from the (a) type of inoculum as compared with the (b) type, which serves as a control. There are occasional anomalous results, as usually occurs in biological work, for which various explanations might be advanced. The impossibility of making identical intramuscular injections can readily be demonstrated by the injection of dyes or suspensions of carbon particles into the opposite breast muscles of fowls, as far as possible in exactly the same way. Subsequent exposure of such injection sites shows that occasionally the inoculum on one side may be delivered mainly between the pectoral muscles while on the other side it may be retained within the muscle substance. It is easy to realize that such considerations may influence the opportunity for local growth of injected tumor cells. The muscular movements of the bird may make it difficult for clumps of cells introduced into the smooth, interfascial plane between the pectoral muscles to become attached and establish a vascular stroma. Occasionally tumors occur at the axillary end of the intramuscular plane, from inoculations made several centimeters below this site. There has never been any evidence, however, that material injected on one side of the sternum has any tendency to spread to the opposite side. From the etiological point of view, posi-

tive results following inoculation of material as illustrated in Chart I are the most difficult to assess. The alternative possibilities are that such tumors have grown from surviving cells in the inoculum, or from cells of the host infected by a virus present in the inoculum, or that both processes may have contributed to the growth of the tumor.

Capillarity Filtration

The isolation of virus from chemically induced tumors might fail owing to its lability within the time taken in the preparation of cell-free filtrates. To meet this type of criticism, a simple method of capillarity filtration introduced by Bedson and Bland (3) for the isolation of viruses and for determining their electrical charge, has been employed. Strips of sterile No. 1 Whatman filter paper 1 × 6 cm., dipped in suspensions of disintegrated tumor, draw up the fluid by capillarity against the action of gravity. The virus of Rous sarcoma 1 can be separated by this method from tumor cells and from red blood cells, which rise in 1 minute to a height of about 1 cm. from the surface of free fluid and form a visible upper boundary, above which cell-free fluid will rise approximately to a further 3 cm. in the course of 5 minutes. Such experiments have not been prolonged for more than about this time, as the method was designed for rapid isolation of virus. Evaporation, which might be inimical to the virus, is checked by conducting the experiment in a moist atmosphere. Impregnated paper strips are cut into centimeter lengths, which are rolled up and inoculated intramuscularly with aseptic precautions into fowls. The upper sections of paper are cut and inoculated first, to avoid the risk of accidental contamination by tumor cells. In the case of the Rous sarcoma, tumor growth has followed the introduction of paper sections at all levels to which the fluid will rise; but in the case of the G.R.C.H. 15 sarcoma, no positive results were obtained with sections above the level to which the red cells will rise. The paper inoculation causes a massive macrophage and fibroblast reaction in every case and one might suppose that this would provide proliferating cells susceptible to infection by any sarcoma virus that might be present. Table V shows the difference between the results obtained with these two types of tumor.

Table V: Recognition of Virus by Capillarity Filtration

Type of tumor	Height from free fluid (cm.)					
	Cells +		Red cell limit	Cells −		
	0-1	1-2		2-3	3-4	4-5
Rous sarcoma 1	6/6	10/10		5/5	4/5	3/4
G. R. C. H. 15 sarcoma	0/1	5/21		0/12	0/10	0/11

Results expressed as fractions: No. of tumors / No. of birds inoculated

Radiation Experiments

It is well established that viruses are much more resistant to x-rays and gamma rays of radium than are animal cells. Baker (1) showed that the virus of Rous sarcoma 1 in aqueous suspension was destroyed by beta rays in much the same way as are bacteria. The resistance of the virus in desiccated Rous sarcoma, submitted to gamma rays *in vitro* and subsequently injected in the usual way into fowls, is shown in Table VI. Doses of the order of 20,000 r are greater than the maximum doses used in clinical treatment, but they fail to destroy the infective agent of the Rous sarcoma *in vitro*.

Table VI: Rous Sarcoma Desiccate Irradiated *in Vitro*

γ-Ray dose	Fowl no.	Site of inoculation	Quantity	Result
15,000 r	1711	Right pectoral muscles	1 cc 1:20 in H_2O	Killed 34th day; large tumors in both breasts
10,000 r	"	Left " "	" " "	
15,000 r	1712	" " "	" " "	Killed 34th day; large tumor in right breast, no tumor in left.
10,000	"	Right " "	" 1:2,000 "	
15,000 r	1713	" " "	" 1:20 "	Killed 34th day; tumor 1.5 cu. cm. in right breast; none in left.
15,000 r	"	Left " "	" 1:2,000 "	
18,000 r	1727	Right " "	" 1:20 "	Killed 30th day; huge tumor in both breasts, visceral secondaries.
18,000 r	"	Left " "	" 1:200 "	
20,000 r	1729	Right and left " "	" 1:20 "	Killed 36th day; large tumor in both breasts.
20,000 r	1730	" " " " "	" " "	Killed 30th day; huge tumors in both breasts, visceral secondaries.

It is not surprising, therefore, to find that radium or x-ray treatment is frequently unsuccessful in birds bearing virus tumors. The picture is complicated by the fact that such tumors not infrequently undergo spontaneous cure and leave the birds immune to further inoculation of virus.

If birds that are developing a natural immunity to the virus have their tumors irradiated, these may retrogress completely. In more susceptible birds, however, irradiation of a virus sarcoma is followed by a peculiar

type of geometrically, rather than anatomically, disposed recurrence. The irradiated tumor may be arrested or even retrogress, but around the edge of the irradiated area little outpost tumors appear within a month and death from visceral metastasis soon follows. In Tables VII to IX a number of results with lished. The recurrent tumors preserve the characteristics of their original strain. The mechanism of this peculiar type of recurrence is thought to be as follows. Radiation acting on the sarcoma cells arrests their division and leads to the destruction of some or all of them, but not of the virus, which is liberated into the

TABLE VII: IRRADIATION OF FOWL SARCOMAS *in Vivo*

Type of sarcoma	Fowl no.	Dose	Site of tumor	Result
McIntosh 5/2	1518	11,470 r γ-rays in 24 hrs.	L. wing	Killed 14 days after treatment. Tumor stationary. Multiple marginal recurrence after irradiation at edge of irradiated area.
		Nil	R. wing	Large tumor on point of ulceration; wing paralyzed.
McIntosh 5/5	1556	11,000 r γ-rays in 21 hrs.	R wing	Killed 42 days after treatment. Tumor slightly smaller. Geometrical secondaries both sides of elbow; visceral metastases.
McIntosh 5/11	1614	4,000 r γ-rays in 20 hrs.	R. wing	Killed 18 days after treatment. Tumor had grown rapidly from 1 cm. in diameter to 7 cm.; soft and hemorrhagic. Secondary in liver.
McIntosh 5/11	1615	12,000 r γ-rays in 42 hrs.	R. wing	Killed 31 days after treatment. Tumor had spread diffusely over whole wing.
Fujinami	16A	3,100 r γ-rays in 120 hrs.	R. leg	Died of pneumonia 11 months after treatment. Tumor in leg smaller and very hard. Tumor had spread to other side of leg as hard mass 2 cm. in diameter; histologically mainly fibrosis; grafts grew slowly but retrogressed.
Fujinami	18	4,800 r γ-rays in 20 hrs.	R. leg	Died 2 days after end of treatment. A very small tumor was found just above tibio-tarsal joint.
		6,500 r γ-rays in 148 hrs.	R. breast	A small tumor was growing from lower pole of breast tumor, which had shrunk during treatment. Histologically tumors were typical Fujinami.
G. R. C. H. 13/6	897	11,750 r γ-rays in 51 hrs.	L. wing	Tumors retrogressed. Fowl died 30 months after treatment without recurrence.
		Nil	L. pectoral	
G. R. C. H. 13/10	1340	11,000 r γ-rays in 22 hrs.	L. wing	Tumor retrogressed. Immune to regrafts 6 months later. Died 14 months after treatment, without tumor.

TABLE VIII: SARCOMA CELLS IRRADIATED *in Vitro* WITH GAMMA RAYS

Type of tumor	Fowl no.	Dose	Site of inoculation	Result
McIntosh 5	1527	approx. 10,000 r	Right wing	Killed 21 days after grafting. Right wing, firm tumor. Left wing, larger, softer, more hemorrhagic tumor.
		Nil	Left wing	
"	1528	10,000 r	Right wing	Killed 14 days after grafting. Right wing, tumor 1 cm.; left wing, tumor 3 cm.
		Nil	Left wing	
"	1545	10,000 r	Left pectoral	Killed 30 days after grafting. Tumor 9 × 5 × 3 cm. Extensive metastases.
"	1546	10,000 r	Left pectoral	Found dead 34 days after grafting. Tumor 13 × 6 × 5 cm. Metastases in lungs.

Rous 1, Fujinami, and McIntosh 5 sarcomas are collected. These experiments have been necessarily conducted on a small scale and over a considerable number of years, but are thought to be worth recording because they offer a practical method of differentiating virus tumors from apparently nonvirus tumors *in vivo*. Geometrical recurrence has been observed in each of the 3 available virus sarcoma strains, but not in sarcomas of the G.R.C.H. series. This type of recurrence was observed by Professor S. L. Baker, apparently for the first time, after irradiation of minute Rous sarcomas with beta and gamma rays from a radon applicator at the time of some joint experiments with the author, but the observation, made in 1925, was not then pub- tissues. The free virus cannot excite new growth in the irradiated area, the cells of which are temporarily inhibited from dividing. The virus then spreads either directly through the tissue spaces, or by the blood stream, until it encounters proliferating mesoblastic tissues at the reacting edge of the irradiated area. Some of these cells are susceptible to infection and give rise to the recurrent tumors. Recent experiments with Rous sarcoma suggest that this geometrical type of recurrence is due to blood-borne virus. Birds were inoculated in the pectoral muscles with Rous sarcoma and at varied intervals thereafter had one or both wings irradiated with 4,000 to 10,000 r (x-rays). In some of these birds geometrically grouped outpost

tumors developed in the same manner as occurred when tumors in the wing were directly irradiated (Figs. 15 to 22 and Table IX). It is proposed to publish a detailed account of these experiments elsewhere.

Irradiation of Tissue Cultures

Tissue cultures of Fujinami sarcoma or G.R.C.H. 15 sarcoma, and of adult fibroblasts, prepared and maintained by Dr. Shukoff, each received doses of gamma rays up to 23,000 r. Though further growth was not observed in tissue cultures, these "dead" cultures of Fujinami sarcoma reproduced the disease when reinoculated into 2 fowls, and from the resulting tumors the virus could again be recovered. Similarly treated cultures of G.R.C.H. 15 sarcoma gave rise to no tumors on reinoculation. Control cultures of this tumor also frequently failed to give positive results. Normal fibroblast cultures never gave rise to any detectable proliferation in inoculated birds.

These irradiation experiments are in some ways analogous to those in which desiccated tumors are used, as in both cases the cells are destroyed by means that do not inactivate viruses. Moreover, in both cases, the whole of the tumor material can be inoculated without fear of transmitting living cells. This point is of importance when considering the possible size of any hypothetical virus that might be retained by the various types of filter commonly employed in preparing "cell-free" tumor extract. The size of inoculated virus is not likely to affect the results of injecting irradiated or desiccated tumors. Thus the available evidence from irradiation experiments lends no support to the idea that chemically induced fowl sarcomas contain an essential virus (Tables VII to X).

Table IX: Rous Sarcomas Irradiated *in Vivo* with X-Rays

Fowl no.	Site of tumor	Site of irradiation	Dose	Result
1704	L. wing	L. wing	2,500 r	Killed 34 days after treatment, 47 days after injection of Rous sarcoma. Tumor diminished but spread to back of wing at edge of irradiated area. Secondaries in inflamed lachrymal and salivary glands, and in lungs.
1757	R. and L. pectoral	L. wing	6,000 r	Killed 188 days after treatment, 26 days after injection of Rous sarcoma. Large tumor in breast. Small tumors at borders of radionecrotic area in wing.
1752	R. pectoral	R. wing L. wing	10,000 r 6,000 r	Dead 4 months after treatment of right wing, 1 month after treatment of left wing, 3 months after injection of Rous desiccate. Small tumor in breast, multiple visceral secondaries. Small tumor in right wing, multiple small tumors in left wing.
1309	L. pectoral	L. wing	4,000 r	Killed 32 days after treatment, 34 days after injection of Rous sarcoma. Large tumor in left breast. Visceral metastases. Small secondaries in left wing.
1312	R. pectoral	L. wing	4,000 r	Died 31 days after treatment, 29 days after injection of fresh Rous sarcoma. Large tumor in breast spreading to air sacs and lungs, secondaries in liver. Geometrical metastases in left wing.

Tumor Inhibitors

Against the use of whole tumor preparations in the search for viruses, it can be argued that inhibiting substances inoculated along with virus might render it harmless. By serial aqueous extractions of disintegrated Rous sarcoma 1, and other "filtrable" tumors, Claude (4)[1] showed that relatively soluble inhibiting substances were present in the first extract, and that consequently a second extract was often more infective than the first. Such double extraction of G.R.C.H. 15 sarcomas yielded 6/7 progressively growing tumors with the first extract, and only 1/7 with the second.

In extensive experiments on similar lines Murphy and Sturm (13) showed that various methods, employed by them to enhance the infectivity of spontaneous fowl tumors of low virulence, failed to yield any evidence of virus infectivity when applied to their dibenzanthracene fowl sarcomas.

Remote Tumors in Birds Injected with Carcinogens

In his first paper dealing with tar-induced fowl tumors, McIntosh (10) drew attention to the high proportion of these in birds injected with tar as compared with the very low incidence of spontaneous tumors in some 5,000 birds that had been under observation for variable lengths of time. He considered that this high incidence excluded the possibility that such tumors could be of spontaneous origin, although most of them

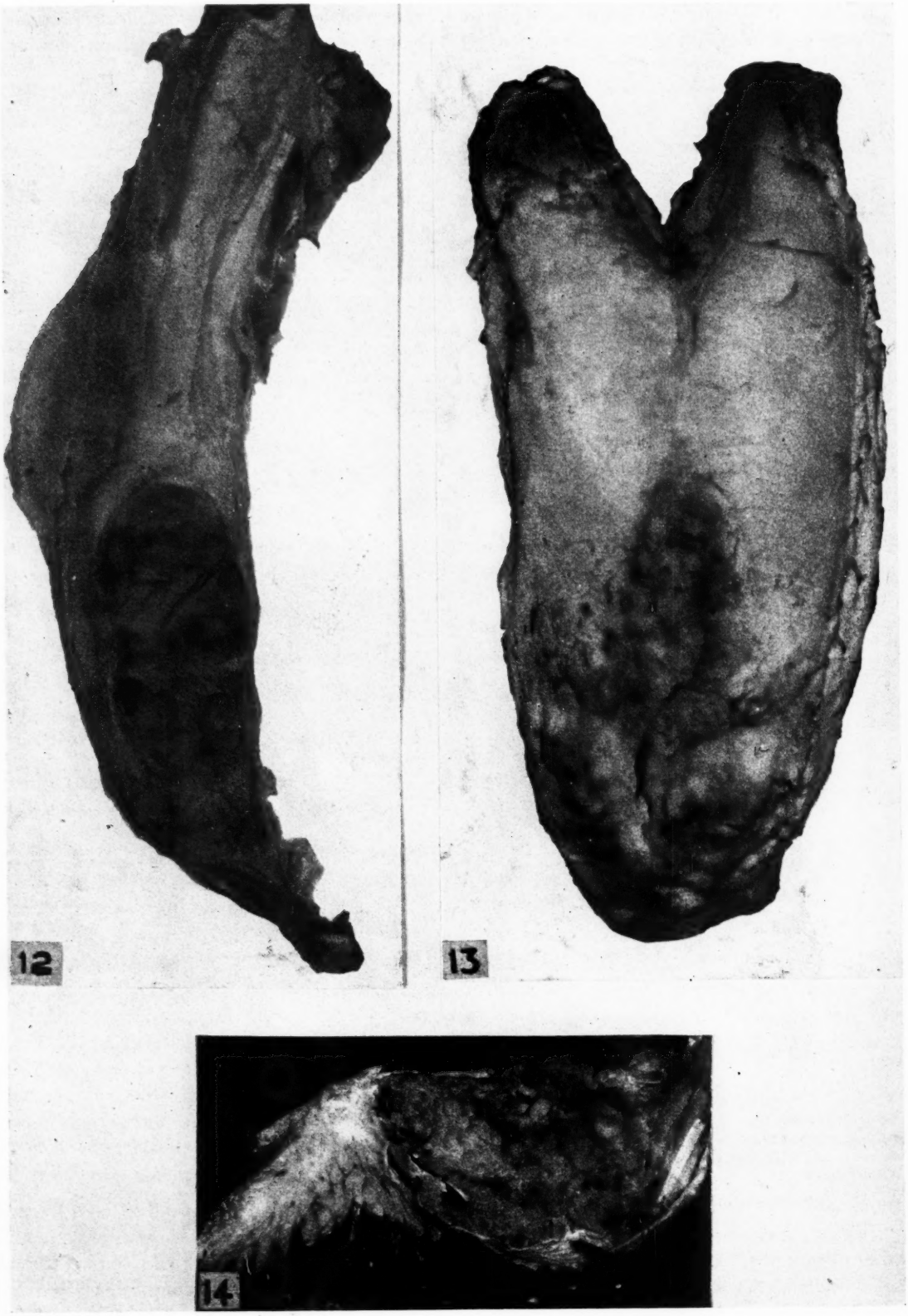
[1] See also, Murphy, J. B., and Sturm, E. Properties of the Causative Agent of a Chicken Tumor. IV. Association of an Inhibitor with the Active Principle. J. Exper. Med. **56**:107-116. 1932.

occurred at sites remote from the injected tar and after very variable latent periods. Remote carcinogenesis is now well recognized, but unless large numbers of control birds are kept under observation for periods as long as those under experiment, it is impossible to assess the risk of spontaneous tumor development. Periods of observation of less than a year in young fowls are practically useless as controls. In the experiments that form the basis of this paper, birds injected with carcinogens have been kept under observation for as long as possible, in some cases for 10 years, and tumors have occurred at the site of injection of carcinogens after latent periods varying between 2 months and $2\frac{1}{2}$ years (17). Birds used in other types of experiment have rarely been kept under observation for more than a year, for reasons of economy and space. Although it has not been feasible to keep large numbers of controls until they died of natural causes, the flock of Brown Leghorns maintained at the Edinburgh Institute of Animal Genetics serves as a partial control to these experiments, and it is known that the incidence of neoplastic disease, including leukoses, is very low (9). Some light may be thrown on this aspect of the problem by considering data relating to all birds

TABLE X

Length of observation	*Nature of inoculum	Tumor incidence population at risk: Barred Plymouth Rocks	Rhode Island Reds	Brown leghorns	White leghorns	No. with local tumors only	No. with similar local and remote tumors	No. with dissimilar local and remote tumors	No. with remote tumors only	Comments
Up to ½ yr.	(a)	5/39	0/2	0/39		5				(a) 4 Tar sarcomas, nos. 69, 140, 147, 158. 1 Dibenzanthracene sarcoma, no. 412. (All Barred Plymouth Rocks.)
	(b)	0/53		0/32						
	(c)	75/367	7/25	219/777	2/32					
" " 1 "	(a)	21/33	0/7	0/10		8	11	1	3	(a) 11 Tar sarcomas, nos. 52, 55, 64, 73, 131, 132, 139, 142, 145, 152, 154. 9 Dibenz. sarcomas, nos. 102, 108, 111, 114, 117, 119, 124, 418, 423. 2 Leukoses, nos. 105, 421 (local injections dibenz.) 1 Remote spindle cell sarcoma, No. 155 (local injection of tar). (All Barred Plymouth Rocks.)
	(b)	2/31		0/6		2				(b) 1 Leukosis, no. 312, and 1 reticulosarcoma, 319.
	(c)	16/83		18/208	1/2					
" " 1½ yrs.	(a)	11/11		1/12		8	4	1		(a) 6 Tar sarcomas, nos. 61, 65, 68, 143, 144, 156. 6 Dibenz. sarcomas, nos. 103, 107, 109, 120, 130, 1971 (brown leghorn). 1 Leukosis, No. 68 (local tar sarcoma). (All (Barred Plymouth Rocks except 1971.)
	(b)	0/2		0/9						
	(c)	8/29		0/68	0/1					
" " 2 "	(a)	9/12		0/11		7	2			(a) 8 Tar sarcomas, nos. 57, 72, 74, 134, 136, 141, 151, 159.
	(b)	0/2		0/3						
	(c)	0/8		1/13					1	1 Dibenz. sarcoma, no. 126. (All Barred Plymouth Rocks.)
										(c) 1 Local sarcoma with leukosis of liver, no. 1252 (brown leghorn).
" " 3 "	(a)	7/18		1/13		7			1	(a) 4 Tar sarcomas, nos. 54, 58, 70, 138. 3 Dibenz. sarcomas, nos. 106, 123, 125.
	(b)	0/6		0/6						
	(c)	0/5		1/9	0/6					1 Dibenz. reticuloendothelioma, no. 1984 (brown leghorn). (All Barred Plymouth Rocks except 1984.)
" " 4 "	(a)	6/9		0/6		4		1	1	(a) 4 Tar sarcomas, nos. 57, 63, 66, 71. 1 Dibenz. sarcoma, no. 100. 1 Ovarian carcinoma, no. 110 (local injection of dibenz. in breast). (All Barred Plymouth Rocks.)
	(b)			0/2						
	(c)	0/1		1/7						(c) No. 897, G. R. C. H. 13/3, cured by radium (brown leghorn).
" " 5 "	(a)	1/2		0/4					1	(a) No. 317, spindle cell sarcoma of back, 4 years after implantation of radioactive thread in liver (Barred Plymouth Rock).
	(b)			0/7						
	(c)									
" " 6 "	(a)	0/2								
	(b)			0/1						
	(c)	0/1								
" " 7 "	(a)		1/1	0/1					1	(a) No. 205, ovarian carcinoma, 7 years after local injection of dibenz. in breast muscle (Rhode Island Red).
	(b)									
	(c)	1/3								(c) No. 381. Spindle cell sarcoma invading liver, which contains a small cholangioma. Origin of sarcoma uncertain (Barred Plymouth Rock).
" " 8 "	(a)	0/1		0/1						
	(b)			0/2						
	(c)									

* (a) Recognized chemical or physical carcinogens.
(b) Other substances.
(c) Grafts or extracts from chemically induced fowl sarcomas. Metastases in this group occurred occasionally, but as most birds were killed before their tumors were advanced, they are not recorded.



FIGS. 12-14

that have been under observation in these laboratories, grouped according to the type of experiment and length of observation. The results (Table X) show that remote tumors are less uncommon in older birds, but their type and distribution do not seem to justify the conclusion that they are related to previous injections of known carcinogens, whether or not these induce tumors at the site of injection.

DISCUSSION

Spontaneous fowl tumors occur in the same apparently capricious manner as do the tumors of other species, and in their natural history give no hint that they differ from them fundamentally. Moreover, as judged by the usual criteria of morbid anatomy and histology, they are similar to the spontaneous tumors of mammals. Only when attempts are made to transmit them experimentally do the peculiar features of some avian neoplasms of mesoblastic origin become apparent. The viruses of some sarcomas and leukoses can be recognized with certainty only by the biological test of inoculation of material derived from neoplastic cells into susceptible hosts. Thus it is uncertain whether the infective agents were intrinsic or extrinsic in relation to the tumors from which they were originally extracted. The experimental pathologist becomes in a sense the intermediate host for the sarcoma virus.

The demonstration by Ellerman and Bang in 1908 that fowl leukosis could be transmitted to other fowls, not only by the inoculation of leukemic blood or tissues but also by cell-free filtrates (6), seems to have been the first positive evidence for an extracellular infective cause of a neoplastic disease. The discoveries by Rous and his colleagues in 1910 (20), and by Fujinami (8) about the same time, that some fowl sarcomas could be transmitted either by cells or by cell-free filtrates are too well known to need recapitulation, but it should be pointed out that Rous was careful to use the noncommittal term "infective agent" to describe the cell-free principle. However, in view of the widespread use of the term "virus" at the present day to cover such diverse pathogenic agents as those of vaccinia and of plant mosaic diseases, there seems to be no objection to talking of infective, cell-free tumor agents as "viruses," provided that no definite biological status (*i.e.*, living or nonliving) is thereby implied. There is general agreement that tumor viruses never cause epidemics of tumors amongst flocks of birds, and that proof of their infectivity depends upon experimental inoculation from a pre-existing tumor. In the case of a spontaneous sarcoma that proves to be "filtrable" we have, therefore, to consider 3 possibilities: (a) the virus was present in the bird before some sequence of events gave it the opportunity to induce new growth in a susceptible tissue; (b) the virus was fortuitously inoculated from some extrinsic source; or (c) the virus is a product of the tumor. Hypothesis (a) offers no explanation of the origin or nature of the virus; (b) suggests an independent existence for the virus, which might be a living organism; (c) limits the origin and the possible nature of the virus. Against objections to the conception of a ubiquitous virus that becomes pathogenic only under special circumstances, it may be pointed out that the flora of the large intestine give no evidence of pathogenicity under normal conditions, though capable of causing fatal peritonitis as a result of injury or disease. In fact, either of the 3 suggested explanations of the relationship of virus to tumor appears to be logically possible, and there may be other valid theoretical explanations. The difficulty, therefore, is to devise crucial experiments to test such hypotheses.

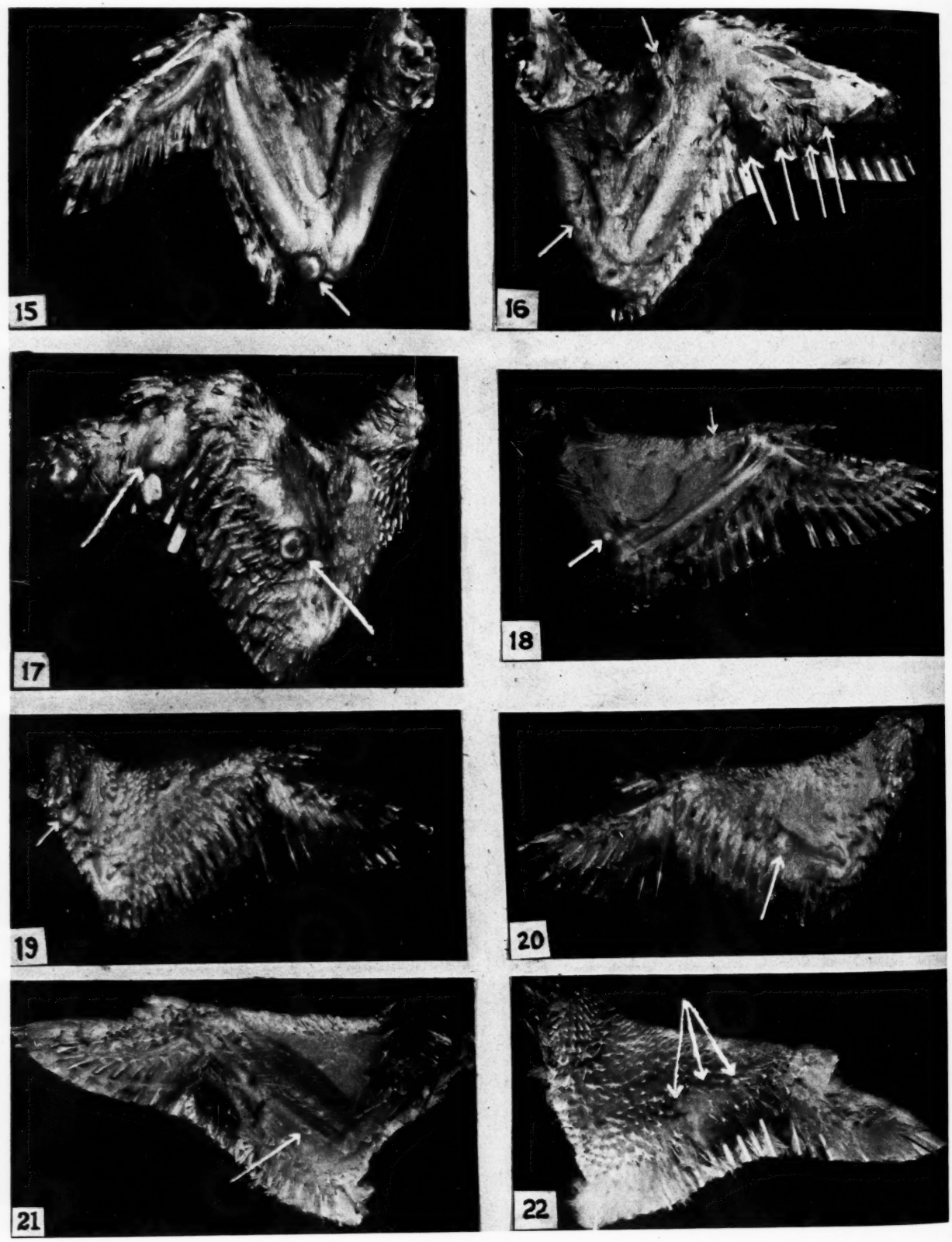
The desire to find a common cause for all malignant tumors led many investigators to seek for agents similar to the virus of Rous sarcoma 1 in mammalian tumors, as well as in other fowl tumors; but despite the discovery of several more "filtrable" tumors, no convincing evidence for the existence of causal viruses in the "nonfiltrable" majority of tumors has been adduced. There is, indeed, much evidence against such a conception. It is not even the case that all avian tumors contain demonstrable causal viruses. In a review of the experimental work on transmissible fowl tumors up to 1933 Claude and Murphy (5) stated that more than 40 spontaneous tumors of the fowl were investigated in the laboratories of the Rockefeller Institute, but that only 4 proved to be transplantable and, later, transmissible by cell-free filtrates. Including tumors reported from other laboratories, some 27 tumors of various types had been transplanted, and 7 altogether had proved "filtrable" at the time of their review. Foulds (7) reviewed and discussed critically the experimental evidence then available, and con-

DESCRIPTION OF FIGURES 12 TO 14

FIG. 12.—Fowl 431. G.R.C.H. 3/2. Injected with cell suspension 106 days previously. Tumors have grown from multiple foci, giving characteristic mosaic appearance.

FIG. 13.—Fowl 1510. McIntosh 5, control. Inoculated both breasts 28 days previously. Note vascularity of tumor, particularly at periphery.

FIG. 14.—Fowl 1614. Right wing, ventral aspect, 18 days after radium treatment (4,000 r) of McIntosh 5. Tumor grafted in right wing 6 days before treatment.



FIGS. 15-22

cluded that no positive evidence was known for supposing the presence of virus in the majority of tumors; but in the case of spontaneous fowl sarcomas he found that viruses could usually be demonstrated in those tumors that could be transplanted. However, the enormous majority of spontaneous fowl tumors are never investigated, and of the few that have been studied in our laboratory none could be transmitted by any of the usual means, including direct cell grafts, so that their etiology remains obscure.

The advent of chemical carcinogens offered the possibility of inducing fowl tumors under conditions that would preclude the introduction along with the carcinogen of any virus similar to that of Rous sarcoma 1. The known tumor viruses are easily inactivated by heat, and cannot survive the temperatures used in bacterial sterilization. The carcinogenic hydrocarbons, on the other hand, are mostly prepared at temperatures over 300 ° C., and therefore must be free from such viruses unless accidental contamination occurs. Early attempts to induce fowl tumors with tar were reviewed in previous publications on this subject (16, 17), and it is sufficient to recall that none of the transplantable tumors so obtained could be transmitted by cell-free filtrates or desiccates.

Our experience has been consistent in this respect, during the 15 years throughout which chemically induced tumors have been under investigation in these laboratories. Thus there is a striking difference in regard to the general transmissibility of the chemically induced and spontaneous fowl tumors; in fact, Murphy and Sturm (13), in their recent account of further experiments with dibenzanthracene fowl sarcomas, state that all transmissible spontaneous fowl tumors that have been "properly investigated have given evidence of containing a transmissible cell-free agent." Nevertheless, they have again failed to find any evidence for the existence of infective agents in 62 induced fowl sarcomas, 12 of which were transplantable. This would seem to suggest that those spontaneous fowl tumors that are easily transmissible are so by virtue of their virus content. It certainly does not prove the viral nature of all spontaneous fowl tumors. It is now clear that transmissible fowl sarcomas, induced by chemical carcinogens that are known to induce similar tumors in mammals, resemble the latter in their demonstrable characteristics, but differ strikingly from the transplantable spontaneous fowl sarcomas in regard to transmissibility by cell-free agents. These results lend no support to the conception that all tumors, or even all fowl tumors, contain a causal virus. On the other hand, McIntosh (10) described in 1933 a series of tumors in birds that had been injected with tar dissolved in lard, some of which he considered as chemically-induced, filtrable tumors. In later experiments with Selbie (11), further tar sarcomas were obtained and, in all, 5 of their 10 induced sarcomas proved to be filtrable at some stage in the course of propagation. Murphy and Sturm (13) reserve acceptance of these results in view of their own and other investigators' consistent failure to confirm them. Careful analysis of McIntosh and Selbie's published result (10, 11, 21), however, shows that the apparent differences between their work and that of other investigators are differences in classification rather than in experimental results. They include in the category of tar tumors not only those originating at or near the site of injection, but also visceral tumors associated with leukoses and differing histologically from the fibroblastic proliferations or tumors induced locally by the tar.

Most other workers have limited their descriptions of chemically induced fowl sarcomas to those occurring in close relation to the injected carcinogen. Some remote tumors occurred in my experiments (Table X), but these were considered unsatisfactory for etiological studies, and have not previously been discussed. Even if these are examples of remote carcinogenesis, they differ in so many respects from each other and from locally induced sarcomas that their significance cannot at present be assessed. If, therefore, the local tar tumors in McIntosh and Selbie's series are considered first, No. 4 (first series, 1933) and Nos. 6 to 10 (second series, 1939) form such a group. Of these tumors, No. 6 could not be transmitted by cell inoculations or by Berkefeld filtrates; Nos. 4, 7, and 10 were transmissible by cell inoculation but not by filtrates; Nos. 8 and 9 were transmissible by both cell inoculation and filtrates. Thus Nos. 4, 6, 7, and 10 are similar to those described by other investigators and contained no demonstrable virus, even when treated with trypsin or by other procedures found by Selbie and McIntosh (21) to enhance the virulence of filtrable tumors. Nos. 8 and 9 therefore merit

DESCRIPTION OF FIGURES 15 TO 22

(See Table X)

FIG. 15.—Fowl 1752, right wing, ventral aspect.
FIG. 16.—Fowl 1752, left wing, ventral aspect.
FIG. 17.—Fowl 1752, left wing, dorsal aspect.
FIG. 18.—Fowl 1757, left wing, ventral aspect.
FIG. 19.—Fowl 1757, right wing, dorsal aspect.
FIG. 20.—Fowl 1757, left wing, dorsal aspect.
FIG. 21.—Fowl 1312, right wing, ventral aspect.
FIG. 22.—Fowl 1312, right wing, dorsal aspect.

special consideration, in view of their filtrability. No. 8, a spindle cell sarcoma, could not be transmitted by intramuscular inoculation of cells, but at the first passage intraperitoneal inoculation was followed in 1 out of 4 birds by several intraperitoneal tumors composed of "densely packed leukemic cells, with a very fine stroma . . . . no evidence of sarcoma could be found" (10). These are well illustrated, and bear no resemblance to the original spindle cell tar sarcoma of No. 8 strain. At the second passage, again, intramuscular inoculation of cells was unsuccessful, but intraperitoneal inoculation from the leukemic tumor was followed in 1 of 3 birds by death 679 days later, with multiple tumors in the liver and 1 in the ovary. These tumors also are illustrated, and described as showing a mixture of myeloid and connective tissue elements in intimate association. In subsequent passages intramuscular inoculations gave spindle cell sarcomas "indistinguishable from the primary tumour," free from leukemia, and filtration experiments were successful on more than one occasion. There seems to be little justification for relating such diverse tumors to the initial injection of tar.

Tumor No. 9, a fibrosarcoma, was successfully inoculated by the intramuscular route, and at the fourth and subsequent serial passages was transmitted by trypsinized Berkefeld filtrates on several occasions. The histology of this tumor strain apparently showed no striking fluctuations but "at the second passage became less differentiated, and consisted of loosely-packed, long spindle cells, with considerable myxomatous degeneration"—features that were retained in later passages. Its characteristics seem to be very similar to those of Rous sarcoma or the Fujinami sarcoma.

Of the remaining remote tumors Nos. 2, 3, and 5 in the first series were filtrable and call for special consideration. The bearer of the tumor that gave rise to No. 5 strain was not injected with tar, but was inoculated, apparently unsuccessfully, from No. 4 strain. No. 5 tumor, therefore, cannot be considered as a tar sarcoma. The tumors from which strains 2 and 3 originated had no obvious relation to the site of tar injection in the breast muscle, though fibroblastic reactions, possibly representing early sarcomas, were present. Tumors in both cases were found in the liver, and were associated with leukemia. Strain No. 2 during subsequent passages was a filtrable fibrosarcoma of the Rous 1 type, while No. 3 was of angiomatous or fibroendotheliomatous type.

McIntosh and Selbie were unable to demonstrate filter-passing causal viruses in Mellanby's dibenzanthracene fowl sarcoma or in my G.R.C.H. 13 tar sarcoma, though they describe several positive results following the inoculation of "standard sand and paper-pulp filtrates" (11). Sand and paper pulp filtrates are included in this paper in the probably cell-free category (Chart I). One positive result occurred in a fowl injected with a filtrate of G.R.C.H. 15 sarcoma, through a filter of 1 inch of sand and 1 inch of packed cotton wool, at the 11th serial passage, but 7 similar tests gave negative results. Occasional anomalous results such as this are thought to be evidence of technical imperfection rather than of true infectivity of the tumor extract. It might be, however, that some apparently trivial difference in procedure would account for striking differences in results between laboratories employing what seem to be similar technics.

Against this possibility is the fact that the McIntosh 5 sarcoma, grown from desiccated tumor kindly supplied by Professor McIntosh, was easily transmissible by filtrates and desiccates in this laboratory and has behaved in all ways like the virus group of tumors and unlike the chemically induced tumors, from which it is also readily distinguishable both macroscopically and histologically. It resembles the Fujinami sarcoma more closely than the Rous 1, but could usually be distinguished from them (Tables II and III).

Thus it is evident that technical considerations alone do not adequately explain failure or success in transmitting fowl tumors, whether chemically induced or not. The association with leukemia in the filtrable tumors of McIntosh's first series can, however, be correlated with the established facts about leukosis in fowls. Oberling and Guérin (14, 15) and others have shown that spontaneous leukemia in fowls can give rise to tumor strains when blood or leukemic tissues are injected intramuscularly or intraperitoneally. It seems, therefore, that such tumors must be regarded either as spontaneous and unrelated to the experimental procedure (injection of tar), or that they are examples of remote carcinogenesis analogous to the induction of lung adenomas in mice following painting with tar, or the remarkable remote tumors induced by Strong in NHO mice injected with methylcholanthrene (22). Judged by any standard, they form a separate group from the local spindle cell sarcomas that usually follow the intramuscular injection of tar and that all investigators are agreed are usually "nonfiltrable." McIntosh and Selbie's tumor No. 9 remains the one example of an apparently uncomplicated filtrable spindle cell sarcoma directly related to the injection of tar. This tumor seems to resemble Rous sarcoma 1, according to the published description, and it seems possible that this type, however it may originate, is characterized by "filtrability." It is suggested that despite the superficial resemblance between all spindle cell fowl sarcomas, the particular myxomatous type, of which Rous 1 and Fujinami sarcomas are the best known examples, should be re-

garded not as typical of cancer in general but as a distinct type, one of whose properties is transmissibility by cell-free extracts, and another a tendency to spontaneous retrogression in experimentally infected birds. It seems possible that the malignancy of such tumors is not irreversible, but depends on the continued existence of the virus, which, so to speak, goads the cells on to useless multiplication. Inactivation of the virus would account for the retrogression of such tumors. In the case of tumors induced with cell-free material, destruction of the cells such as occurs in radiation therapy is not necessarily followed by immunity to re-infection with the virus; so that in cases of retrogression of virus tumors one must suppose that there is developed an immunity to the virus. Reversible malignancy of this kind, which can occur in some chemically induced tumors also, *e.g.,* in rabbits painted with carcinogenic hydrocarbons if the painting is not prolonged (2), cannot easily be reconciled with the conception of somatic mutation. The great majority of clinically recognizable human cancers, however, behave as irreversibly malignant tumors. Histological methods do not yet differentiate between potentially reversible and irreversible malignancy, though retrogressing tumors, of course, can be distinguished from progressively growing ones. Irreversible malignancy may supervene in the course of tumor growth, whether this was initiated by a virus or by chemical carcinogens, and if this change is due to somatic mutation the continued growth of the tumor would be independent of the initial stimulus, whatever its nature. Thus in the virus tumors, persistence of the virus may not be essential for their continued growth, whereas the transmissibility of the tumor by cell-free material must depend on its persistence.

As the practical interest of all cancer research is in the bearing it may have on the etiology of human cancer, the results of experimental irradiation of virus-induced and chemically induced tumors are thought worthy of further attention. The type of geometrically arranged recurrence described above as characteristic of 3 virus-induced fowl sarcomas, has not so far been observed in chemically induced fowl tumors or in experimentally transmitted mammalian tumors, and is certainly not commonly seen in human cancer. It is hoped to extend this type of investigation to other virus tumors and to spontaneous tumors of animals, as it offers the possibility of comparing the response of animal and human tumors under similar conditions and might throw some light on the etiology of the human disease.

Those who support the conception that all malignant tumors are due to viruses seem to be agreed that more than one virus type is concerned. If this be so, any common cause of cancer must be something beyond the virus and common to more than one type of virus. It might, in fact, be any agent or sequence of events capable of causing a particular type of somatic mutation. There seems no reason, therefore, to postulate that such a common factor can only be shared by different viruses, and no reason to postulate the existence of viruses as causal agents of those tumors in which repeated search gives uniformly negative results.

SUMMARY

Experience in transmitting chemically induced fowl sarcomas during the period 1935 to 1945 is described. A new sarcoma, G.R.C.H. 15, induced with 1,2,5,6-dibenzanthracene and propagated serially in pedigreed Brown Leghorn fowls of known genetic interrelationship, has, like earlier chemically induced sarcomas (G.R.C.H. 1 to 14), resisted all attempts to extract from it any infective causal virus.

Comparisons between sarcomas G.R.C.H. 1 to 15 and 3 virus sarcomas revealed slight but definite histological differences between the two groups. Analysis of 30 generations of serial passage of G.R.C.H. 15 sarcoma revealed a genetic basis for the resistance to tumor inoculation frequently encountered throughout these experiments. The seasonal variation in transmissibility previously reported for sarcomas G.R.C.H. 1 to 14 was also observed in the case of G.R.C.H. 15 sarcoma.

The classification of tumor extracts is discussed and a method of obtaining cell-free extracts by capillarity described. Typical results of irradiation of virus tumors and their extracts are described. Virus sarcomas show a tendency to recur in the margin of an irradiated area, differing in this respect from chemically induced fowl sarcomas and from transplantable mammalian sarcomas.

All the experimental results serve to emphasize the differences between our chemically induced fowl sarcomas and the virus sarcomas derived from fowls with spontaneous tumors or leukoses. Assertions that causal viruses in chemically induced fowl sarcomas have been demonstrated are analyzed and discussed. Only one such tumor (sarcoma No. 9 of McIntosh and Selbie) can be accepted as a virus-transmissible sarcoma derived from a tar sarcoma of apparently similar histology. This tumor seems to resemble closely the group of filtrable sarcomas of spontaneous origin.

A theoretical explanation of some apparently conflicting results is advanced, and the conclusion drawn that there is no reason to postulate a single virus or a group of closely allied viruses as the essential cause of all forms of cancer.

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Abstracts

Reports of Research

The Neoplastic Potentialities of Mouse Embryo Tissues. I. The Findings with Skin of C Strain Embryos Transplanted to Adult Animals. ROUS, P., and SMITH, W. E. [Rockefeller Inst., New York, N. Y.] *J. Exper. Med.*, **81**:597-620. 1945.

The authors have devised a method whereby the epidermis of mouse embryos, implanted in the voluntary muscles of adults of the same inbred (C) strain, can be selectively exposed to the action of a chemical carcinogen. It was found that the encysted epidermis underwent neoplastic changes with great regularity and rapidity under the influence of methylcholanthrene. The growths that arose from the embryo epidermis, papillomas and carcinomas, were like those appearing on the skin of adult mice after exposure to methylcholanthrene.—D. S.

The Neoplastic Potentialities of Mouse Embryo Tissues. II. Contributory Experiments; Results with the Skin of C3H and Webster-Swiss Embryos; General Considerations. SMITH, W. E., and ROUS, P. [Rockefeller Inst., New York, N. Y.] *J. Exper. Med.*, **81**:621-646. 1945.

Attempts to show that an agent out of the blood would localize in inflammatory lesions of the skin failed. Neoplastic changes arose only occasionally in skin of C3H and Webster-Swiss mouse embryos, transplanted with methylcholanthrene into the muscles of adults of these strains. The skin of new-born and suckling mice proved resistant, while that of their mothers and of young adults was susceptible, to the carcinogenic effect of methylcholanthrene. Gastric carcinomas, which can be elicited only after months when ordinary carcinogenic technics are applied to adult mice, arose rapidly and regularly in stomach fragments from C strain embryos, implanted, together with the carcinogen *OSM* (olive oil containing 1% of methylcholanthrene and 1% of Scharlach R), into the muscles of adult mice of the same strain. Tumors from fragments of the lung, ovary, and bile passages have been similarly produced. The implications of the findings in papers I and II are discussed. The results do not support the idea that carcinogenesis of the transplanted embryo tissue is due to the lodgment therein of tumor-producing viruses.—M. H. P.

Methylcholanthrene Squamous Cell Carcinoma of the Rat Prostate with Skeletal Metastases, and the Failure of the Rat Liver to Respond to the Same Carcinogen. DUNNING, W. F., CURTIS, M. R., and SEGALOFF, A. [Wayne Univ. Coll. of Med., and Detroit Inst. for Cancer Research, Detroit, Mich.] *Cancer Research*, **6**:256-262. 1946.

Squamous cell carcinomas of the prostate were induced by 3 mgm. pellets of compressed methylcholanthrene crystals in 1 of 9 Fischer line 344 and in 10 of 17 A×C line 9935 rats that survived an average of 241 and 329 days, respectively, after the pellets were implanted in the organ. One of these induced tumors had generalized peritoneal and skeletal metastases. The litter sisters of these males, 9 Fischer line 344 and 15 A×C line 9935 females, survived an average of 257 and 528 days, respectively, after similar pellets were implanted in the parenchyma of the liver but failed to develop any liver neoplasms. A study of the history after subcutaneous transplantation of one of the induced prostate carcinomas showed that it regularly metastasized to the lungs, superficial lymph nodes, and skeleton. Five successful transplants of a mammary adenocarcinoma to the prostate were followed by weekly x-ray examinations, but no skeletal involvement was observed.—Authors' abstract.

Copper and Zinc in Epidermal Carcinogenesis Induced by Methylcholanthrene. CARRUTHERS, C., and SUNTZEFF, V. [Barnard Free Skin and Cancer Hosp., and Washington Univ. Sch. of Med., St. Louis, Mo.] *J. Biol. Chem.*, **159**:647-651. 1945.

Hyperplastic mouse epidermis contained about 45% less copper and 30% less zinc than did normal epidermis. A transplantable squamous cell carcinoma contained even smaller quantities of copper and zinc than did hyperplastic epidermis. Judged from the mineral composition, the sequence is normal to hyperplastic tissue to carcinoma.—H. J. C.

Calcium, Copper, and Zinc in the Epidermal Carcinogenesis of Mouse and Man. CARRUTHERS, C., and SUNTZEFF, V. [Barnard Free Skin and Cancer Hosp., and Washington Univ., St. Louis, Mo.] *Cancer Research*, **6**:296-297. 1946.

Data are presented to show that the process of epidermal carcinogenesis in mouse and man is similar with respect to calcium, copper, and zinc. Normal mouse epidermis contained almost three times as much calcium as did normal human epidermis, about twice as much zinc, but about the same amount of copper as did normal human epidermis. Methylcholanthrene-treated mouse epidermis was quite similar chemically to normal human epidermis, which agrees somewhat with their histology. The calcium, zinc, and copper contents of a transplantable squamous cell carcinoma were 80, 67, and 83% less than those of normal mouse epidermis, while these 3 metals in human squamous cell carcinomas were respectively, 47, 30, and 70% less than in normal human epidermis.—Authors' abstract.

Potassium and Calcium Content of Gastric Carcinoma. BRUNSCHWIG, A., DUNHAM, L., and NICHOLS, S. [Univ. of Chicago, Chicago, Ill.] *Cancer Research*, **6**:230-232. 1946.

Microfilm copies of such papers here abstracted as are available may be obtained from Medicofilm Service of the Army Medical Library at 25¢ for each complete article, not exceeding 25 pages in length—and 10¢ for each additional 10 pages or fraction thereof. Prepayment is not requested. Remittance may be made with subsequent orders and in such manner as found most convenient. Address—Medicofilm Service, Army Medical Library, Washington, D. C.

In human material, gastric carcinomas were found to contain relatively less calcium and more potassium than the adjacent uninvolved gastric mucosa. In a benign papilloma of the stomach situated between 2 separate carcinomas the K/Ca ratio was of the same order as in the carcinomas and differed in like manner from the K/Ca of the surrounding uninvolved mucosa.—Authors' abstract.

Potassium and Calcium Content of Carcinomas and Papillomas of the Colon. DUNHAM, L., NICHOLS, S., and BRUNSCHWIG, A. [Univ. of Chicago, Chicago, Ill.] *Cancer Research,* **6**:233-234. 1946.

In human material, carcinomas of the colon were found to contain less calcium and more potassium than adjacent normal mucosa. In papillomas of the colon an increase in potassium of the same order as that found in carcinomas was observed, but the degree of reduction in calcium was not so pronounced as in the latter.—Authors' abstract.

Experimental Studies on the Significance of Glutamic Acid for the Growth of Malignant Tumors. RØRDAM, H. N. K. [Univ. Inst. of Path. Anat., Copenhagen, Denmark] *Acta path. et microbiol. Scandinav.,* **21**:480-498. 1944.

Twenty mice of the Street strain bearing Crocker sarcoma 180 transplants received *l*(+)-glutamic acid, as a suspension containing 2 gm. in 10 ml. of 0.1540 *M* NaH-glutaminate, in 4 subcutaneous injections of 0.2 ml. at intervals of 2 days beginning 2 days after transplantation. The average tumor weight 2 days after the last injection was 0.35 gm., as compared with 0.50 gm. in controls bearing the sarcoma transplants but receiving no glutamic acid. In another experiment, the results of which were judged to be statistically not significant, both *l*(+)-glutamic acid and *d,l*-glutamic acid reduced the average weight of the tumors by about the same amount. The findings are discussed in connection with Kögl's theory of the role of glutamic acids in tumor growth.—M. H. P.

Les sécrétions internes du cancer du sein. [The Internal Secretions of Cancer of the Breast.] LOEPER, M., and LOEPER, J. [Paris, France] *Presse méd.,* **52**:17-18. 1944.

Among 26 women with carcinoma of the breast, instances of high basal metabolism, hypercalcemia, and functional uterine bleeding were present. Water-soluble extracts of 3 tumors produced a rise in basal metabolic rate in rabbits. Water-soluble extracts of 2 tumors gave rise to hypercalcemia when injected into dogs. Fat-soluble extracts of 2 of 4 tumors had an estrogenic effect in ovariectomized rats.—C. A.

Age and Dosage in the Induction of Breast Cancer in Mice by the Mouse Mammary Tumour Agent. DMOCHOWSKI, L. [Lab. of Imp. Cancer Research Fund, London, England] *Brit. J. Exper. Path.,* **26**:192-197. 1945.

A high incidence of breast cancer was induced by administering dried mammary tumor tissue of mice of the A and RIII strains to 4 month old hybrid female mice (C57×A), whose mammary glands were completely developed in the absence of the mammary tumor agent. With the dried RIII tumor tissue the cancer incidence was 83% at an average age of 18 months, with the A tissue it was 60% at 20 months. A similar tumor incidence resulted when 4 month old hybrids were given 12 times the amount of dried tumor tissue as was given to 4 week old hybrids.—R. J. L.

Comparative Potency of the Mammary Tumor Agent of Mice of Different Genetic Constitution. DMOCHOWSKI, L. [Lab. of Imp. Cancer Research Fund, London, England] *Brit. J. Exper. Path.,* **26**:267-269. 1945.

Breast cancer was induced by dried mammary tumor tissue of mice of: (1) RIII strain in C57×C3H hybrids and Ax mice; (2) C3H strain in C57×C3H and C57×A hybrids and Ax mice; (3) A strain in C57×C3H hybrids. The tumor-producing potency of the dried RIII tumor tissue (as shown by the breast tumor incidence and average tumor age) is greater than that of dried C3H tumor tissue, while dried A tumor tissue is the least potent. Whether the potency of the agent varies in different strains, or whether tumors of different strains contain varying amounts of the same agent requires further investigation.—R. J. L.

Relationship of the Inherited Susceptibility and the Inherited Hormonal Influence in the Development of Mammary Cancer in Mice. BITTNER, J. J., and HUSEBY, R. A. [Univ. of Minnesota Med. Sch., Minneapolis, Minn.] *Cancer Research,* **6**:235-239. 1946.

During the course of this study mammary tumors were not found in any of the descendants of the *fostered* females of the cancerous A and Z (C3H) stocks and their reciprocal hybrids, mice that were susceptible to mammary cancer but did not have the milk agent. Observations made upon breeding females of the cancerous A and Z stocks and their reciprocal F_1 and F_2 hybrids were in accord with the theory that the mice of these 2 strains may have the same inherited susceptibility to mammary cancer. The difference in the incidence of mammary cancer in the virgin females of the A and Z strains was due to gene action in controlling some hormonal mechanism. This effect has been called the inherited hormonal influence, and is probably the result of the action of multiple genes. The same genes probably do not produce the inherited susceptibility and the inherited hormonal influence. The characteristics (concentration and/or activity) of the milk agent may differ in the A and Z stocks and possibly influenced the incidence of mammary cancer that was observed in the hybrids when they were maintained as virgins. These differences were not apparent in the breeding hybrids. Each inciting influence in the genesis of mammary cancer in mice may be almost completely determining in its effects, and thus equally important.—Authors' summary.

A Comparative Study of the Ovaries of Virgin Mice of the dba and C57 Black Strains. FEKETE, E. [Roscoe B. Jackson Memorial Lab., Bar Harbor, Me.] *Cancer Research,* **6**:263-269. 1946.

A comparative study of the right ovaries of virgin dba and C57 black mice revealed the following differences: (a) The average number of ova escaping from one ovary at a given ovulation is 5.2 in dba, and 4.2 in C57 black mice. Since the growing follicles are the source of estrogen, it can be presumed that this hormone is pro-

duced in larger quantity in the dba mice. (b) In C57 black virgin females 3 sets of corpora lutea are the most that can be found in an ovary. In the ovaries of dba mice the corpora lutea persist for a longer period, and 7 or more sets may be present. Consequently it can be presumed that a larger quantity of progesterone is produced in the ovaries of the dba mice. (c) In older animals hyaline changes occur in the lutein cells of persistent corpora in dba mice, and in some ovaries large hyalinized areas are present; this alteration does not occur in the ovaries of C57 black mice. (d) Groups of yellow lipochrome cells originating in corpora lutea atretica undergo atrophy and become dispersed in the ovarian stroma of dba mice, whereas in C57 black mice these cells hypertrophy and form aggregated nodules. (e) Ovarian cysts occur more frequently in dba mice (30.7%) than in C57 black mice (13.4%).—Author's abstract.

A Transplantable Cornifying Squamous-Cell Carcinoma in Mice. ENGELBRETH-HOLM, J. [Univ. Inst. of Path. Anat., and Danish Anti-Cancer League's Cancer Research Lab., Copenhagen, Denmark] *Acta path. et microbiol. Scandinav.*, **21**:418-422. 1944.

By numerous passages, a transplantable carcinoma originating from squamous cell epithelium in mice has been transformed from a keratinizing to a basal cell type and has been rendered more virulent. Photomicrographs illustrating the change are presented.—M. H. P.

Factors Influencing the Stability of a Filtrable Agent of Chicken Leukosis and Sarcoma. GOTTSCHALK, R. G. [Cornell Univ. Med. Coll., New York, N. Y.] *Cancer Research*, **6**:270-277. 1946.

A filtrable agent of leukosis and sarcoma of chickens (agent 13) was sedimented from tumor extracts at a speed of 15,000 r.p.m. in an angle centrifuge. When resuspended and diluted in buffer, the sediments contained only a small fraction of the infectivity of the original extracts. The total activity was recovered when the sediments were kept in concentrated solution, or resuspended and diluted in buffer containing inactivated rabbit serum. A preparation of agent 13, purified by 3 successive centrifugations at 15,000 r.p.m., was infectious in amounts containing 8.6×10^{-8} mgm. of nitrogen. The 50%-response-dose (calculated from latent period values) contained 3.2×10^{-7} mgm. of nitrogen. Inactivated supernatants of extracts from sarcoma 13 did not afford a better protection than solutions of inactivated serum. Inactivated extracts of a nonfiltrable chicken tumor (sarcoma 16) had no inhibitory action on agent 13. It was thus shown that protein solutions have a nonspecific protective action on preparations of agent 13.—Author's abstract.

On the Cultivation of a Mouse Leukosis in Vitro. BICHEL, J. Dissertation. *Univ. Press of Aarhus, Denmark.* 1939, 103 pp., 165 refs. 10 plates.

The literature on the behavior of blood elements in tissue culture is reviewed, and a point is made of the fact that all attempts to cultivate lymphocytes unaltered for any considerable length of time have failed. Similarly it has been impossible to find cells in leukemias that would behave as many malignant cells do and grow indefinitely *in vitro*. The author describes his attempts to culture a transmissible mouse leukosis. It was found that the leukosis cells would show continued growth only when provided with the support of other cells, and for this purpose he cultured fibroblasts of chick embryo satisfactorily. The leukosis cells invaded the peripheral portions of the fibroblast colony and there multiplied. By transferring such mixed cultures, the *in vitro* cultivation of the leukosis cells was kept up for $4\frac{1}{2}$ months and probably could have been continued indefinitely. They never transformed to other types, and when inoculated into mice, reproduced the original leukosis. The author feels that these results substantiate the correctness of the concept that mouse leukosis is a neoplastic disease.—K. P.

Synovial Sarcoma and Normal Synovial Tissue Cultivated *in Vitro*. MURRAY, M. R., STOUT, A. P., and POGOGEFF, I. A. [Columbia Univ., and Presbyterian Hosp., New York, N. Y.] *Ann. Surg.*, **120**:843-851. 1944.

Cultures from 3 human synovial sarcomas revealed two main elements: flattened, spindle-shaped cells and membranous or epithelioid cells, both of which showed fibrinolytic activity and were associated with the formation of reticulin and refractive vacuoles. The predominant type of cell may not be the same *in vivo* and *in vitro;* this is also true of mesotheliomas. Macrophages were seen quite late, and fibroblasts appeared in older cultures. Studies of normal synovial cultures indicated that the synovial cell is a specific cell.—W. J. B.

Some Cultural and Cytological Characteristics of Human Tumors *in Vitro*. ROYLE, J. G. [Bryn Mawr Coll., and Lankenau Hosp. Research Inst., Philadelphia, Pa.] *Cancer Research*, **6**:225-229. 1946.

Four human tumors, 3 carcinomas of the breast and 1 adenoma of the thyroid, were grown in roller tube cultures and subcultured in hanging drop preparations for cytological study. Growth patterns of fibroblasts and epithelial cells were typical of those previously reported except for the early formation of acini in an adenoma of the thyroid and the development of whorls of cells at the peripheries of the zones of outgrowth in 2 carcinomas of the breast. The separation of the whorls from the zones of outgrowth and the subsequent proliferation of new cells from the whorl-like clusters, suggest a similarity to the metastatic behavior of malignant epithelial cells *in vivo*. Cytological examination showed stroma cells of the tumors to be bipolar or multipolar cells with broad or long oval nuclei. Spherical mitochondria predominated in epithelial cells, rod-like and filamentous mitochondria in fibroblasts. Neutral red granules were found in perinuclear arrangements and scattered in the cytoplasm of both types of cells. The cytoplasm of epithelial cells of carcinoma cultures was generally dense and diffusely granular; however, epithelial cells of the adenoma and fibroblasts of the adenoma and the 3 carcinomas of the breast were homogeneous with localized granular areas. Binucleate and trinucleate cells were seen in carcinoma cultures.—Author's abstract.

Tissue Responses to Physiologically Active Substances. THOMSON, B. F. [Connecticut Coll., New London, Conn.] *Bot. Rev.*, **11**:593-610. 1945.

One section of this article reviews the literature on the relation of plant growth substances to crown gall. The histological similarity of auxin-induced tumors to crown galls and the fact that plant reactions that follow application of auxins also accompany gall formation suggest that crown gall development is associated with increased auxin content. Measurements of the auxin content of galls have not yet established whether they actually contain more auxin than normal tissues. However, experiments with attenuated *Phytomonas tumefaciens* show that growth substances are somehow involved in gall formation. In these experiments the attenuated bacteria by themselves fail to produce galls, but if extra growth substance is supplied to the infected tissue large galls develop.—R. B.

Colchicine and X-rays in the Treatment of Plant and Animal Overgrowths. LEVINE, M. [Montefiore Hosp., New York, N. Y.] *Bot. Rev.*, **11**:145-180. 1945.

This review presents a summary of work on the effects of colchicine and x-rays on tumors. Plant tumors produced by *Bacterium tumefaciens* are not prevented from developing, but when they are well formed they may be destroyed by colchicine. Some animal tumors regress or grow at a retarded rate following colchicine injections, but others appear not to be affected. Experiments having to do with the effect of colchicine on the sensitivity of animal tumors to x-rays have likewise given variable results. The drug in some instances appears to render tumors more sensitive to irradiation and in others to have no effect. This article also reviews the literature on the nature of the changes produced in cells by colchicine and x-rays, and summarizes the applications of colchicine in genetics and endocrinology.—R. B.

Experimental Imitation of Tumour Conditions. THOMAS, P. T. [John Inness Horticultural Inst., Merton, London, England] *Nature, London*, **156**:738-740. 1945.

A consideration of certain experimental data led to the following conclusions: The change from a normal to a malignant cell is of the nature of a mutation. This might be brought about by an increase in the amount of heterochromatin, or by a mutation in nuclear genes, or in cytoplasmic plasmagenes. The malignant cell is characterized by increased nucleic acid metabolism and more rapid mitosis. A comparable condition is said to be brought about in yeast cells by the action of camphor and chemical carcinogens. The increased production of lactic acid by malignant cells brings about changes in the physical state of their cytoplasm, which results in abnormal spindle development and consequent nuclear aberrations. Similar disturbances of the spindle mechanism can be induced with lactic acid in the root-tip tissue of rye.—R. J. L.

Rare Cervical Tumors. HAASJES, C. H. [Shelby, Mich.] *J. Am. Vet. M. A.*, **105**:146. 1944.

It was observed that during the last month of pregnancy of a cow, 8 fleshy tumors extended from the cervix through the vulva. These were removed with local anesthesia. Delivery was uneventful, and there was no recurrence. No sections of these rare tumors were made.—E. E. S.

Melanosarcoma in a Senior Yearling Heifer. ANON. *J. Am. Vet. M. A.*, **105**:30. 1944.

A photograph of the tumor *in situ* is presented. The tumor was said to be a melanin-impregnated sarcoma near the udder, a rarity in young animals of the bovine species. The cow was observed for several years; no metastases or recurrence developed. Photomicrographs of the tumor are not given.—E. E. S.

Clinical and Pathological Reports

Clinical investigations are sometimes included under Reports of Research

General

Beitrag zur Frage von der Teratomgenese. Die morphogenetische Theorie. [On the Genesis of Teratoma. The Morphogenetic Theory.] HOLMDAHL, D. E. [Upsala Univ., Upsala, Sweden] *Acta path. et microbiol. Scandinav.*, **19**:603-620. 1942.

A review and discussion, with an extensive bibliography. The author's morphogenetic theory embraces the blastomere-, the parthogenesis-, and pole-cell-theory. The organizer-theory is rejected as not clear and not directly applicable to the entire vertebrate organism.—M. H. P.

Etiology

A Discussion of the Causes of Cancer. REIMAN, S. P. [Lankeneau Hosp. Research Inst., Philadelphia, Pa.] *Rhode Island M. J.*, **28**:573-578; 615-616. 1945.

A general discussion for practitioners, touching upon biological factors, hormones, genetics, carcinogens, and viruses.—M. E. H.

Heredity

Multiple Neurofibromatose, eine erbliche Krankheit mit Übergängen in andere Geschwulstformen (Gliomatose, Spindelzellensarkom, Hämangiosarkom). [Multiple Neurofibromatosis, a Hereditary Disease with Transition to Other Forms of Tumors (Gliomatosis, Fusocellular Sarcoma, and Hemangiosarcoma).] HARBITZ, F. *Acta path. et microbiol. Scandinav.*, **19**:448-481. 1942.

Multiple neurofibromatosis (von Recklinghausen's disease) appeared as a dominant hereditary disease in 4 generations of a family observed for 37 years. One boy of the third generation, who had multiple neurofibromas in practically every nerve, died at the age of 9 years of gliomatosis (or neurinomatosis) spreading in the meninges especially along the spinal cord and medulla oblongata, and on the base of the brain. A girl of the third generation died at the age of 23 of an intrathoracic tumor that had developed in a nerve trunk. In many of the neurofibromas in this case there were hemangiomas with

transition to hemangiosarcomas, which had produced metastases in the meninges and elsewhere. The large intrathoracic tumor consisted in part of tissue of this kind and in part of a fusocellular sarcoma.—M. H. P.

Epithelioma Adenoides Cysticum. Whittle, C. H. *Proc. Roy Soc. Med.*, **38**:502. 1945.

Report of a case, with biopsies, in an agricultural laborer whose mother and niece were affected in the same way. —E. L. K.

Diagnosis—General

Über den Nachweis von Tumorzellen in Sternalpunktaten. [The Detection of Tumor Cells in Sternal Puncture Specimens.] Gormsen, H. Report to Danish Path. Soc., Nov. 24, 1941, from abstr. in *Acta path. et microbiol. Scandinav.*, **19**:312-313. 1942.

Tumor cells were found in 56 of 311 sternal puncture specimens taken from 263 patients with cancer; *i.e.*, 18% of the punctures permitted diagnostic inferences. Sternal puncture revealed tumor cells in only 1 of the 21 patients with sarcoma, in neither of 2 with reticulum-cell sarcoma, and in 28 of 198 patients with carcinoma; 21 of these 28 patients showed metastases to bone on x-ray examination, and 3 others of the 28 subsequently developed such metastases. In 2 cases of lymphosarcoma, sternal puncture revealed marked infiltration of the bone marrow by lymphatic elements. Of 16 myeloma patients, 14 showed myeloma cells in the sternal marrow, while the other 2 showed such cells only elsewhere in the skeletal system.—M. H. P.

Therapy—General

Neurosurgical Procedures for the Relief of Pain in Advanced Cancer. Crutchfield, W. G. [Univ. of Virginia Med. Sch. and Hosp., University, Va. [*Radiology*, **45**: 253-259. 1945.

There is a wide field of usefulness for neurosurgery in the relief of intractable pain due to cancer. The defeatist attitude should not be assumed until the possibilities of relief by neurosurgery are investigated. The results of chordotomy, particularly upper thoracic, are good. Alcohol injection and posterior rhizotomy have not proved very successful in the author's experience. Section of cranial nerves, particularly the trigeminal, or injection of its peripheral branches with alcohol may give relief from pain originating from tumors of the mandible, maxilla, tongue, lips, and paranasal sinuses. Prefrontal lobotomy or a similar operation may possibly, in the future, prove valuable for improving the patient's mental state.—R. E. S.

The Treatment of Malignant Tumors by Bacterial Toxins as Developed by the Late William B. Coley, M.D., in the Light of Modern Research. Nauts, H. C., Swift, W. E., and Coley, B. L. [New York, N. Y.] *Cancer Research*, **6**:205-216. 1946.

At least 15 preparations of "Coley's toxins" have been used since the method was introduced in 1892, 3 being apparently more potent than the rest, and the technic of administration has varied considerably as regards site, dosage, frequency, and duration of treatment. Successful results have been obtained by a considerable number of physicians as well as by Coley himself; the results were not always published. Because of greater strength of available preparations, and use of a more effective technic, the method was more effective in the early years. Still better results may now be possible by use of purified and concentrated preparations administered with the most effective technic, adapted to the stage of the disease and to the type of neoplasm. More than 600 case histories have been abstracted in detail, and end results are tabulated for 484 cases in which the diagnosis was histologically confirmed. Among 312 inoperable cases there were 190 five year survivals; among 132 operable cases there were 106 five year survivals. Excluded from tabulation were 80 cases lacking microscopic confirmation, 55 instances of giant cell tumors (determination of malignant ones difficult), and 32 cases in which complete regression occurred but which were followed less than 5 years. Reasons are given to explain why the method has not achieved wider recognition in the past.—Authors' abstract.

Radiation

The Practical Use of Radium in Dermatology. Morse, J. L. [New York, N. Y.] *Urol. & Cutan. Rev.*, **49**: 576-578. 1945.

Nevus vasculosus, angioma cavernosum, verruca plantaris, and keloids are conditions in which the use of radium has given satisfactory results. The author advocates low dosages and adequate shielding. Epitheliomas, keratoses, verruca vulgaris, and corns are more suitable for surgery, or surgery and radiation.—V. F. M.

Radium Therapy of Hemangioma. Freedman, L. M. J., Jacox, H. W., and Beinhauer, L. C. [West. Pennsylvania Hosp., Pittsburgh, Pa.] *Urol. & Cutan. Rev.*, **49**:560-567. 1945.

The radium treatment of 107 hemangiomas yielded "65 perfect results, or 60%." The results were regarded as satisfactory in over 93% of the 95 patients, most of whom were infants. No statement is made regarding the length of time these patients were followed, nor regarding late sequelae except that minimal scar formation was noted in 11 instances and telangiectasis in 1 instance.—V. F. M.

Multiple Cavernous Hemangiomata of the Skin, Brain and Skeleton. Report of Case Treated by Roentgen Rays and Observed for 7½ Years. Pohle, E. A., and Clarke, E. A. [Univ. of Wisconsin Med. Sch., Madison, Wis.] *Urol. & Cutan. Rev.*, **49**:283-287. 1945.

The facts about multiple hemangioma are reviewed. One case is reported in detail in which roentgen therapy has given an excellent result including cessation of jacksonian seizures.—V. F. M.

Roentgen Therapy of Primary Neoplasms of the Brain and Brain Stem. Peirce, C. B., Cone, W. V., Elvidge, A. E., and Tye, J. G. [Roy. Victoria Hosp., Montreal Neurol. Inst., and Faculty of Med., McGill Univ., Montreal, Canada] *Radiology*, **45**:247-252. 1945.

Ninety-one patients with primary malignant neoplasms of the central nervous system were subjected to radiation

over the period from 1939 through 1943. Twenty-five of 60 patients with complete follow-up to September 1, 1944, are "alive and well" or "alive and well save for residual symptoms." No deleterious effects due to the radiation were observed. In most cases regrowth of hair occurred, and in a number of patients longer palliation was obtained than would have been anticipated from the usual course of the disease. There was no direct relationship between the size of the tumor dose and length of survival, but it would appear that the minimum tumor dose for astrocytoma would be greater than 6,000 r and for glioblastoma, greater than 7,500 r. Glioblastoma multiforme continues to have a high mortality rate although x-ray may produce longer survival. The effect on astrocytoma is not so strong as might be expected.

In the discussion of this paper M. Sosman states that a tumor dose of 6,000 r or more will produce damage to the normal brain but that this dose is justifiable in the treatment of a fatal disease. P. Bailey believes that a tumor dose of 5,000 r is as effective as 15,000 r and that higher doses are not justifiable because of damage to the brain.—R. E. S.

Degenerative Effects of Large Doses of Roentgen Rays on the Human Brain. Wachowski, T. J., and Chenault, H. [Univ. of Illinois Coll. of Med., Chicago, Ill.] *Radiology,* **45**:227-246. 1945.

Six cases are presented of brain tumor, treated by high voltage roentgen radiation, which showed definite signs and symptoms of brain damage. The tumor dose in all cases was more than 6,000 r. Autopsies were performed in 3 cases and revealed extensive degenerative changes in nerve tissue. The larger blood vessels did not show a corresponding radiation effect; the doses used failed to destroy all the tumor tissue. Sixty-three references.—R. E. S.

Irradiation Failures in Early Cervical Cancer. Buschke, F., and Cantril, S. T. [Tumor Inst. of Swedish Hosp., Seattle, Wash.] *Am. J. Roentgenol.,* **54**:60-71. 1945.

Statistically, the results of adequate irradiation therapy in cancer of the cervix are superior to those of radical surgery. However, from 20 to 30% of stage I and II cases are not cured by even the most competent and skillful radiologists. The authors review their series of 19 out of 79 patients in stage I and II, either dead or with active disease, to see whether they might have been saved by more efficient irradiation or by radical surgery. The analysis shows 13 irradiation failures due to causes outside the pelvic cancer, 2 definitely due to incomplete irradiation, and 4 unexplained. These 4 patients might have been improved by surgery; however 3 of them were not acceptable for such procedure according to Meigs's rigid standards. The authors conclude that since it is impossible to recognize these cases prior to treatment, the use of drastic surgery on large series of cases in the hope of improving statistics is not warranted. Most of the failures by irradiation would also have been failures by surgery, and some radiation successes would probably not have been surgical successes.—E. H. Q.

Osteogenic Sarcoma and Chondrosarcoma. With Special Reference to the Roentgen Diagnosis. Pendergrass, E. P., Lafferty, J. O., and Horn, R. C. [Hosp. of Univ. of Pennsylvania, and Penn Mutual Life Insurance Co. Foundation for Study of Neoplastic Dis., Philadelphia, Pa.] *Am. J. Roentgenol.,* **54**:234-256. 1945.

All cases of osteogenic sarcoma, chondrosarcoma, and other primary malignant bone tumors, seen in the Hospital of the University of Pennsylvania between 1931 and 1945, were reviewed. In 26 cases, roentgen and pathological diagnosis did not agree. An abstract is presented of each of these cases, with roentgenograms and photomicrographs of most of them. It is concluded that the diagnosis of the pathologic type of malignant bone tumor cannot be made with certainty from the roentgen examination alone. Benign conditions may simulate malignant bone tumors. A complete skeletal survey should be made in every case of bone lesion that is suspected of being malignant.—E. H. Q.

Radiation Therapy of Carcinoma of the Thyroid. Rosh, R., and Raider, L. [Bellevue Hosp., New York, N. Y.] *Radiology,* **44**:556-564. 1945.

The incidence, pathological types, sites of metastases, diagnosis, and treatment of thyroid carcinoma are discussed, and 64 cases from the Radiation Therapy Department of Bellevue Hospital are analyzed.—R. E. S.

Oral Cavity and Upper Respiratory Tract

Ophthalmologic and Neurologic Symptoms of Malignant Nasopharyngeal Tumours. Godtfredsen, E. [Copenhagen, Denmark] *Acta path. et microbiol. Scandinav.,* **Supplement 55**:1-323. 1944.

To assist in the earlier recognition of patients with cancer of the nasopharynx, an analysis is presented of 454 cases of primary nasopharyngeal carcinomas and sarcomas, a group more than double the size of the one that was previously regarded as the largest. The initial symptom was cervical in 32%, nasal in 30%, otologic in 23%, and ophthalmic-neurologic in 16%. An average of 11 months elapsed between the onset of the first symptom and the arrival at a correct diagnosis. Analysis of the clinical picture existing 5 months before diagnosis revealed ophthalmic-neurologic symptoms in 34.8% of the patients. A nasopharyngeal tumor should be suspected in all patients with solitary or multiple cranial nerve paralyses, enlarged cervical glands, nasal stenosis, or tubal occlusion. Despite the occurrence of ophthalmic-neurologic symptoms, metastases to the cervical glands, or considerable destruction of the base of the skull, patients have been freed from symptoms (observation period 5 to 12 years) by intensive radiation therapy. Of the 266 treated patients, 75% were freed from symptoms initially, and 22% remained symptom-free for at least 5 years. Photographs, photomicrographs, a clinical tabulation of all cases analyzed, and a bibliography of 262 references accompany this comprehensive report.—M. H. P.

Twenty Cases of Reticulosarcoma and a Special Form of this Tumor ("with Clear Cells"). With a Histological Examination of 109 Malignant Tumors of Tonsils and Rhinopharynx. Bang, F. Report to Danish Path. Soc., Mar. 27, 1941; from abstr. in *Acta path. et microbiol. Scandinav.,* **18**:439-440. 1941.

Of 109 cancers of the tonsils and nasopharynx, 52 originated from the epithelium of the mucosa, 2 were transi-

tional cell carcinoma, 25 lymphoepithelioma, 3 lymphosarcoma, 20 reticulosarcoma, 1 doubtful reticulosarcoma, and 6 "various other tumors." The reticulosarcomas were dictyocytic or syncytial, and the latter group was divided into an entirely syncytial form and a special form designated as "reticulosarcoma with clear cells." This form of tumor (presumably the special form mentioned last) appeared very sensitive to radiation.—M. H. P.

Tumour of the Left Ethmoid. Formby, M. L. *Proc. Roy. Soc. Med.*, **38**:620. 1945.

Description of a case.—E. L. K.

Intrathoracic Tumors—Lungs—Pleura—Heart

The Proximal Extension of Carcinoma of the Lung in the Bronchial Wall. Griess, D. F., McDonald, J. R., and Clagett, O. T. [Mayo Foundation and Mayo Clin., Rochester, Minn.] *J. Thoracic Surg.*, **14**:362-368. 1945.

Fifty-five cases of carcinoma of the lung were studied to determine the proximal extension of the malignant lesion along the bronchial wall. The authors group all lung carcinomas as either squamous cell, arising from bronchial lining, or adenocarcinoma from the mucous glands and do not segregate any undifferentiated or "reserve cell" form. More of the squamous tumors had extended proximally, but the glandular tumors were seen to extend farther.—E. E. S.

Tumors of the Bronchi. Holinger, P. H. [Univ. of Illinois Coll. of Med., and St. Luke's Hosp., Chicago, Ill.] *J. Indiana M. A.*, **37**:671-673. 1944.

Discussion.—E. E. S.

Adenoma of the Bronchus. Moersch, H. J., Tinney, W. S., and McDonald, J. R. [Mayo Clin., Rochester, Minn.] *Surg., Gynec. & Obst.*, **81**:551-558. 1945.

A clinical-pathological discussion, with a tabulation of data on 38 cases.—J. G. K.

Cylindroma of the Bronchus. Report of Six Cases. McDonald, J. R., Moersch, H. J., and Tinney, W. S. [Mayo Clin., Rochester, Minn.] *J. Thoracic Surg.*, **14**:445-453. 1945.

Six abstracts of clinical findings are presented with descriptions of the specimens obtained by operation or at autopsy. The authors point out that, although benign, these tumors differ from adenomas in a tendency to involve the trachea and in secretion of a mucoid material. Since a myxomatous interstitial tissue was sometimes encountered, a relationship to the mixed tumors of the salivary glands is suggested. There is a brief summary of the symptoms, which are chiefly dependent upon bronchial obstruction and secondary infection.—E. E. S.

Extragenital Choriocarcinoma in the Male. Laipply, T. C., and Shipley, R. A. [West. Reserve Univ., and Univ. Hosps. of Cleveland, Cleveland, Ohio] *Am. J. Path.*, **21**:921-927. 1945.

Seven cases from the literature are reviewed, and an additional case is reported in which surgical removal of a complex teratoma originating in the thorax was followed 7 years later by death from recurrent or independent thoracic teratoma with choriocarcinomatous metastases.—J. G. K.

Über sog. Endokardmyxome. [So-Called Endocardial Myxoma.] Ringertz, N. [Caroline Hosp., Stockholm, Sweden] *Acta path. et microbiol. Scandinav.*, **19**:262-299. 1942.

A report of a case, a review of the literature with an extensive bibliography, and a discussion of the pathogenesis of this condition. The author believes that so-called endocardial myxomas are neither organized thrombi nor tumors derived from connective tissue, but are like an independent growth of mesenchymal cells proliferating in the medium of the circulating blood and comparable to a tissue culture.—M. H. P.

A Case of Metastatic Heart Tumor—Neurofibrosarcoma. Anthun, O. [Norske Diknohjem, and Norske Radiumhosp.] *Acta path. et microbiol. Scandinav.*, **21**:434-440. 1944.

A case report, with photomicrographs and a photograph.—M. H. P.

Diaphragm and Abdominal Wall

Neurofibroma of the Diaphragm. Klassen, K. P., Patton, R., and Bemen, F. M. [Ohio State Univ., Columbus, Ohio] *J. Thoracic Surg.*, **14**:407-413. 1945.

The authors believe this to be a unique case report, although other types of tumors have been reported in this location. The tumor was large and encroached seriously on the capacity of the thorax but caused little dyspnea. A presenting symptom was pain in the upper extremities. The tumor presumably arose from the phrenic nerve, but the latter was not paralyzed.—E. E. S.

Sarcoma of Rectus Muscle: Surgical Management. Pugh, H. L., and Robnett, A. H. [U.S.N.] *U. S. Nav. M. Bull.*, **45**:729-732. 1945.

The literature bearing upon abdominal wall tumors is reviewed. A case of myxosarcoma of the right rectus muscle is reported, and the technic of removal of this tumor and plastic repair of the abdominal wall is described.—C. W.

Leukemia, Lymphosarcoma, Hodgkin's Disease

Essays on the Biology of Disease. Chapter 7. The Biology of Follicular Lymphoblastoma. Moschcowitz, E. [Mt. Sinai Hosp., New York, N. Y.] *J. Mt. Sinai Hosp.*, **11**: 298-299. 1945.

Follicular lymphoblastoma is, in most instances, the forerunner of lymphosarcoma and less frequently of lymphatic leukemia. The disease usually presents a milder course than the conventional lymphosarcoma does and is particularly radiosensitive so that both lymph nodes and spleen may greatly decrease in size after only a few treatments. Unfortunately, the condition does not remain cured. In the majority of cases, the disease returns, sometimes after many years (4 to 15), the lymph nodes become resistant to x-rays, and the patient succumbs.—A. Cnl.

The Brain in Leukemia. A Clinicopathologic Study of Twenty Cases with a Review of the Literature. Leidler, F., and Russell, W. O. [Washington Univ. Sch. of Med., St. Louis, Mo.] *Arch. Path.*, **40**:14-33. 1945.

From a study of 20 brains from patients with myeloid,

monocytic, and lymphoid leukemia and (1 case) leukosarcoma, and an analysis of 47 cases of leukemia from the literature, the authors conclude that approximately 80% of patients with leukemia have significant pathological changes in the brain, consisting of hemorrhages and infiltrations of leukemic cells, that 20 to 35% of leukemic patients show neurologic signs and symptoms, and that approximately 29% die of brain hemorrhage.—J. G. K.

Myeloid Leukemia, Probably Eosinophilic in Character. Hodgson, J. R., Kaump, D. H., Steiner, G., and Bovill, J. [Providence Hosp., and Wayne Univ. Coll. of Med., Detroit, Mich.] *Am. J. Clin. Path.*, **15**:389-394. 1945.

Case report.—J. G. K.

Colchicine in Acute Myelogenous Leukemia. Kneedler, W. H. [Philadelphia, Pa.] *J.A.M.A.*, **129**:272-273. 1945.

In this case of acute myelogenous leukemia, no conclusion can be drawn concerning the beneficial effect of colchicine, which was administered over a 13½ month period until death. However, further trials of the drug are recommended.—M. E. H.

Ulcerative Hodgkin's Disease and Lymph Node Imprints. Sweitzer, S. E., and Winer, L. H. [Univ. of Minnesota, and Minneapolis Gen. Hosp., Minneapolis, Minn.] *Arch. Dermat. & Syph.*, **51**:229-236. 1945.

Report of a case in which imprints made from ulcerating cutaneous lesions revealed characteristic Sternberg-Reed cells.—J. G. K.

Adrenal

Pheochromocytoma: Case Report with Certain Observations on the Pathologic Physiology. Mortell, E. J., and Whittle, J. P. [Univ. of Virginia Sch. of Med. and Hosp., University, Va.] *J. Clin. Endocrinol.*, **5**:396-402. 1945.

This is a case report of a pheochromocytoma of the adrenal medulla with a typical clinical picture. In addition the patient experienced generalized convulsions that could be precipitated apparently by hyperventilation. At operation the tumor was observed to be fixed close to the diaphragm and to be displaced inferiorly upon inspiration. The activity of the pressor substance in the tumor fluid did not decrease over a period of 48 hours, which suggested to the author that the mother substance was not adrenaline but a more stable substance such as tyrosine.—J. B. H.

Pheochromocytoma and Sudden Death Following Injury of the Head. Dolgin, W. *Arch. Path.*, **40**:135-140. 1945.

Case report.—J. G. K.

Pituitary

Neoplastic Diseases of the Human Hypophysis. Kraus, J. E. [St. Francis Hosp., Peoria, Ill.] *Arch. Path.*, **39**: 343-349. 1945.

General review.—J. G. K.

The Relationship of "Crooke's Changes" in the Basophilic Cells of the Anterior Pituitary Body to Cushing's Syndrome (Pituitary Basophilism). Kepler, E. J. [Mayo Clin., Rochester, Minn.] *J. Clin. Endocrinol.*, **5**:70-75. 1945.

The author proposes and discusses the thesis that Crooke's changes (hyalinization) of the basophilic cells of the anterior lobe of the pituitary gland represent an effect and are not a cause of Cushing's syndrome. The changes are held to be secondary to hyperfunction of the adrenal cortex.—J. B. H.

Cushing's Syndrome without Adenomatous or Hyperplastic Changes in the Pituitary Body or Adrenal Cortices and Complicated by Alkalosis: Report of Case with Necropsy. Cluxton, H. E., Jr., Bennett, W. A., Power, M. H., and Kepler, E. J. [Mayo Clin., Rochester, Minn.] *J. Clin. Endocrinol.*, **5**:61-69. 1945.

A case of Cushing's syndrome is reported in which necropsy revealed no tumorous tissue of an endocrine organ or other part of the body and no hyperplasia of the adrenal cortex. The sole anatomical abnormality observed consisted in hyaline changes (Crooke's changes) of the basophilic cells of the anterior lobe of the pituitary gland. Studies of the patient before death showed alkalosis and low concentrations of potassium in the blood plasma. The levels of potassium were increased and existent electrocardiographic changes were influenced by the administration of potassium citrate. It is suggested that the hyaline changes in the pituitary were secondary and that the clinical manifestations were an expression of adrenal cortex hypersecretion, despite the absence of supporting histological evidence.—J. B. H.

Statistics

A Ten Year Report of the Tumor Registry of the Yale University School of Medicine Based on Total Admissions for Cancer to the New Haven Hospital 1935-1944. Macdonald, M. C. [Yale Univ. Sch. of Med., New Haven, Conn.] *Connecticut M. J.*, **9**:687-697. 1945.

The object of the survey was to determine what the cancer situation is in the general hospital, and the report is based on 3,561 tumor records that have been abstracted or revised since 1940. Of the total cases, 1,979 or 55.6% were in males, and 1,582 or 44.4%, in females. The 5 year survivals represent 22.2% and 19.8% of total admissions for males and females, respectively, from 1935 through 1939. Cancer was proved by microscopic examination in 86.5% of the hospitalized males and in 88.8% of the hospitalized females. A study was made of the age of the patients at the time of admission, stage of the disease, site of the lesion, presence or absence of metastasis, and the delay period between the first symptom and the initiation of treatment.—M. E. H.

The Racial Distribution of Cancer. II—Tumors of the Kidney, Bladder and Male Genital Organs. Schrek, R. [Vet. Admin., Hines, Ill.] *Ann. Surg.*, **120**:809-812. 1944.

In this study no significant difference in incidence of renal tumors in white and colored persons was found. Cancer of the penis, scrotum, and prostate occurred more frequently in Negroes, but cancer of the bladder was less frequent in colored men (but not women). Colored men younger than 50 years had a relatively low incidence of cancer of the testis.—W. J. B.